NUTRITIONAL EVALUATION OF SEAWEEDS FOR POTENTIAL DEVELOPMENT OF VALUE-ADDED BISCUITS

MWALUGHA HELTAN MWALUKUMBI

MASTER OF SCIENCE
(Food Science and Nutrition)

JOMO KENYATTA UNIVERSITY OF
AGRICULTURE AND TECHNOLOGY

2022

Nutritional Evaluation of Seaweeds for Potential Development of Value-added Biscuits

Mwalugha Heltan Mwalukumbi

A Thesis Submitted in Partial Fulfilment of the Requirements for
the Degree of Master of Science in Food Science and Nutrition of
the Jomo Kenyatta University of
Agriculture and Technology

2022

DECLARATION

This thesis is my original work and has not been presented for a degree in any other university.

Signature: ………………………………… Date: …………………

Heltan Mwalukumbi Mwalugha

This thesis has been submitted for examination with our approval as the university supervisors.

Signature: ………………………………… Date: …………………

Prof. Joseph G. Wakibia, PhD
JKUAT, Kenya

Signature: ………………………………… Date: …………………

Prof. Glaston M. Kenji, PhD
JKUAT, Kenya

Signature: ………………………………… Date: …………………

Prof. Mwanjala A. Mwasaru, PhD
JKUAT, Kenya

DEDICATION

This work is dedicated to the entire ***Weni Ngoo*** and ***Weni Senga*** clans of Taita-Taveta County, Kenya for their constant source of love, strength, support, inspiration and encouragement. May this work be an inspiration for them to seek knowledge of the unknown.

ACKNOWLEDGEMENTS

I would like to acknowledge the Jomo Kenyatta University of Agriculture and Technology for facilitating this research as well as its Research, Production and Extension Division (Project No. JKU/2/4/RP/134), for the financial support without which the work would not have been possible. I would like to acknowledge my mentors and supervisors, Prof. Glaston M. Kenji, Prof. Mwanjala. A. Mwasaru and Prof. Joseph G. Wakibia for their constant advice, criticism, far-sighted guidance, constructive evaluation and encouragement throughout the entire study.

I would also like to acknowledge Dr. Paul N. Karanja, Mr. David M. Votha, Ms. Jescar Oruka and Mr. David A. Rasugu of Food Science and Technology Department for their devoted dedication in assistance me during the laboratory work. I also acknowledge field assistance from Ms. Mary Mkonu, Mr. Sammy Kadhengi, Mr. Alex Kimathi, Mr. Joseph Kilonzi and Mr. Mwithi Kithusi of Kenya Marine and Fisheries Research Institute, Mombasa.

I would like to express my heartfelt gratitude to my parents, Prof. Livingstone M. Ngoo and Mrs. Edah M. Mwalugha, and my brothers, Davis S. Mwalugha, Granton M. Mwalugha and Oliver M. Mwalugha for their infinite love, support and encouragement throughout years of my studies. God bless you always.

Finally, I am highly indebted to The Almighty God, for the gift of life, good health, wisdom and insight throughout the research work.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF APPENDICES

ABBREVIATIONS/ACRONYMS

AACC	American Association of Cereal Chemists
AAS	Atomic Absorption Spectroscopy
ANOVA	Analysis of Variance
AOAC	Association of Official Analytical Chemists
DMRT	Duncan Multiple Range Test
DNA	Deoxyyriboonucleic acid
DW	Dry weight basis
FAO	Food and Agricultural Organization
GOK	Government of Kenya
IDA	Iron Deficiency Anaemia
JKUAT	Jomo Kenyatta University of Agriculture and Technology
KDHS	Kenya Demographic and Health Survey
KMFRI	Kenya Marine and Fisheries Research Institute
MNM	Micronutrient malnutrition
NEM	Northeast Monsoon
NFE	Nitrogen Free Extract
NFNP	National Food Security and Nutrition Policy
NRC	National Research Council
PPB	Parts per billion
RDI	Recommended Daily Intake
RNA	Riboonucleic acid
RPE	Research, Production and Extension
SEM	Southeast Monsoon
SWM	Southwest Monsoon
TSE	Total Specific Energy
UNICEF	United Nation Children’s Fund
UV-VIS	Ultra Violet Visible Spectrophotometer
WHO	World Health Organization

ABSTRACT

Over the years, the Government of Kenya (GOK) has strived to achieve national, household and individual food security throughout the country. The National Food Security and Nutrition Policy (NFNP) highlights the nutitional effects on a population primarily fed on maize and advocates diversification of eating habits. Seaweeds have been consumed in most Asian countries for their valuable macronutrients such as fibre, protein, lipids, carbohydrates and micronutrients such as vitamins and minerals. In the African continent, seaweeds utilization is limited presumably due to limited knowledge of their nutritional potential. This study adopted a complete random design in sampling and nutritional evaluation of seaweeds. A random complete block design was adopted in the preparation of biscuit and assessment of physical properties and sensory attributes of the baked seaweed biscuits. The aim of the research was to collect selected seaweeds in Kenya and assess their nutritional composition for potential utilization in biscuit development. The seaweed samples were collected and identified from three coastal sites in Kenya (Mkomani, Kibuyuni and Mtwapa) in March, July and October 2013. The sites were selected because they had a wide diversity of seaweed species. The analytical methods by the Association of Official Analytical Chemists (AOAC) were used for proximate analysis whereas mineral composition was determined using atomic absorption spectrophotometry (AAS). The chemical analyses were performed on each species of the seaweed in triplicates. The major proximate component was the nitrogen free extract (NFE) with a mean value of 42.09 ± 0.83% dry weight (dw) whereas the lowest component was crude fat (1.81 ± 0.04% dw). The major mineral element was magnesium with a mean value of 1523.45 ± 66.93 mg/100g dw whereas the lowest component was lead (0.20 ± 0.01 mg/100g dw). The chlorophytes (green algae) had the highest magnesium and calcium contents compared to the phaeophytes (brown algae) and rhodophytes (red algae). The chemical composition of the seaweeds varied significantly among the species, algal divisions, months and sites ($p<0.05$). The levels of cadmium and lead in the seaweeds collected were higher than the recommended limits in food set by World Health Organisation (WHO) due to pollution of seawater as a result of human activities. Considering the daily recommended intake of 4g/day, little seaweed amount could be incorporated in food products such as biscuits. From the study, the seaweed, *Dictyota* sp. 2 had the highest iron (314.25 ± 3.87 mg/100g dw) and zinc (50.69 ± 1.40 mg/100g dw) contents, but the lowest cadmium 0.20 ± 0.04 mg/100g dw) and lead (0.02 ± 0.00 mg/100g dw) contents ($p<0.05$) and therefore could be incorporated in biscuits. Sensory evaluation of seaweed biscuits showed that the scores for the seaweed biscuits (1–5% seaweed) were only slightly lower than the control (0% seaweed). All the seaweed biscuits were of acceptable sensory properties suggesting potential incorporation of seaweeds in biscuits. Howeer, biscuits with more than 3% seaweed content exceeded the permissible limits for cadmium in foods according to Codex Alimentarius Commission (CAC), thus were not recommended due to the risk of toxicity to consumers. Biscuits with 1–3% seaweed content provided recommended daily intake (RDI) levels of 30.58–63.67% for iron and 20.13–25.73% for zinc. However, there was the concern of cadmium accumulation in human beings over time that could result in adverse effects. The findings of this study indicate that seaweeds are rich in nutrients such as protein and minerals, and when incorporated in small amounts in common food vehicles are potential resources for seaweed-based products for improved human nutrition. Further, the Government of Kenya could engage stakeholders in developing a guide in creating awareness of seaweeds in order to develop seaweed-based functional foods for improved health and nutrition.

CHAPTER ONE

INTRODUCTION

1.1 Background information

Over the years, the Government of Kenya (GOK) has strived to achieve national, household and individual food security throughout the country. Sustained efforts and initiatives have been developed and implemented gradually to build self-reliance to reduce chronic food insecurity and address micronutrient deficiencies (GOK, 2009; 2011). The National Food Security and Nutrition Policy (NFNP) provides an overarching framework covering the multiple dimensions of food security and nutrition improvement (GOK, 2011). It has been purposefully developed to add value and create synergy to existing sectoral and other initiatives of government and partners (GOK, 2009). It recognizes the need for multi-public and private sector involvement, and that hunger eradication and nutrition improvement is a shared responsibility of all Kenyans. The policy and associated actions will remain dynamic to address contextual changes and changing conditions over time. This policy is framed in the context of basic human rights, child rights and women's rights, including the universal 'right to food' (GOK, 2009).

Seaweeds or macroalgae have played a vital role in human nutrition and health in the Asian region mainly Korea, Indonesia, China, Japan, Philippines and Vietnam (Ohno, 1993; Trono and Toma, 1993; Tanaka and Nakamura, 2004), and in the South Pacific region mainly Fiji, Vanuatu and Hawaii (Novaczek, 2001). Observational studies done in Asia indicated that seaweeds have potential benefits against diabetes, cancer and cardiovascular disease (Brownlee *et al.*, 2011; Brown *et al.*, 2014).

Currently, about 75% of the total seaweed production is primarily for human food. Seaweed is consumed either dry or fresh for its nutritional value or for flavouring in the form of condiments, soup, salad and dessert (Kilinç *et al.*, 2013). The remaining 25% is used for extraction of major phycocolloids such as carrageenan, alginates and agar for various applications in food, medicine and cosmetic industry (Ohno and Critchley, 1993; Lahaye, 2001; McHugh, 2003); and agriculture, water purification and aquaculture (Pereira and Yarish, 2010; Abreu *et al.*, 2011; Chopin, 2012; Fleurence *et al.*, 2012; Kim *et al.*, 2014). The global demand for macroalgae has been growing due to the increase in usage in food and feed industries. This had led to the development of improved exploitation and cultivation techniques in African and Asian countries (FAO, 2016).

Seaweed farming is mostly in South Africa, Namibia, Tanzania, Madagascar, Mozambique and Kenya (Bolton *et al.*, 2009; Msuya, 2013). Seaweeds are used primarily for extraction of thickening agents mainly agar, alginates and carrageenan (Msuya, 2013). Utilization of seaweeds as food is limited probably due to scanty knowledge of the nutritional composition of the seaweeds (Mwalugha *et al.*, 2015). In Kenya, about 400 seaweed species have been identified and documented (Bolton *et al.*, 2007). However, little attention has been given to their utilization either for domestic or industrial application despite introduction of commercial seaweed cultivation in 2010 (Msuya *et al.*, 2014). Studies on Kenyan seaweeds indicate that they are potential sources of nutrients such as protein, fibre, carbohydrates, lipids, minerals and vitamins (Mwalugha *et al.*, 2015; Muraguri *et al.*, 2016). Muraguri *et al.* (2016) attempted to identify a seaweed from five species in Kenya with the best potential of being utilized in the formulation of chicken sausages. The study showed that chicken sausages with upto 1% seaweed had improved chemical, functional and sensory characteristics with reduced cholesterol levels (Muraguri *et al*., 2016).

Variation of chemical composition of seaweeds is attributed to changes in environmental parameters such as nutrients, salinity, water temperature and light (Dawes, 1998), and ecological conditions (Lobban and Harrison, 1994). The Kenya coast lies in the tropical region where the weather is influenced by the Monsoon winds of the Indian Ocean. The climate and weather systems on the Kenya coast are dominated by the pressure systems of the Western Indian Ocean and the two monsoon periods namely, the Southeast monsoon (from May to October) and Northeast monsoon (November to March) (Mutai and Ward, 2000).

The diverse people of the world are increasingly appreciating the significance of seaweeds to health and nutrition. This has led to the emergence of functional foods and ingredients due to their enhanced health benefits and the potential to decrease the risk of micronutrient deficiencies and diseases, and the need for consumption of low-glycaemic foods. The importance of seaweeds has led to incorporation of seaweeds in food products such as jams, jellies, chocolate, curry, pizza, breakfast bars, beverages (Dettmar *et al.*, 2011; Combet *et al.*, 2013), bread, biscuits (Turan *et al.*, 2011; Jennifer and Kanjana, 2018), sausages, meatballs and frankfurters (Fernández-Ginés *et al.*, 2005).

Biscuits are popular convenient foods worldwide due to varied tastes, easy availability, longer shelf-life and affordable cost (Chavan and Kadam, 1993; Nagi *et al.*, 2012). Biscuits refer to baked product containing flour, sugar, baking soda, shortening and water (Jan *et al.*, 2016). However, other ingredients such as eggs, flavours and other composite flours can be used (Cheng and Bhat, 2016). These ingredients are mixed together to form a biscuit dough (Chevallier *et al.*, 2000) before baking.

Seaweeds have high amounts of crude ash (Chapman and Chapman, 1980; Fleurence, 1999) with appreciable minerals that could be potentially utilized in biscuits to combat micronutrient deficiencies of public health concern. Iron and zinc deficiencies are the commonest public health concern among children and expectant mothers in the world (Brabin *et al.*, 2001a). The occurrence of these deficiencies is due to low-level consumption of iron and zinc-rich foods (Prasad, 1991; Cousins, 1996; Brabin *et al.*, 2001a). In Sub-Saharan Africa, these deficiencies are serious and widespread, affecting mainly the poor particularly expectant mothers and children. In Kenya, fortification of common household commodities such as milled cereal products has contributed to mitigation of iron and zinc deficiencies (WHO, 2016). Iron and zinc are critical components in various functions in the human body. Inadequacy of iron and zinc in the human body increases the risk of morbidity and mortality among expectant mothers and children (Ramakrishnan, 2002; Rolfes *et al.*, 2008; Uchendu, 2011).

1.2 Statement of the problem

Currently, knowledge of the chemical composition of seaweeds has been used to formulate food products to improve their functionality by addressing health-related complications and mitigate deficiencies (Kumar *et al*., 2018). Anaemia is a public health problem associated with, among others, impaired cognitive and motor development in children and increases the risk of mortality for the mothers and neonates (Brabin *et al.*, 2001b). In Kenya, the prevalence of anaemia, iron deficiency and iron-deficiency anaemia in expectant mothers is 41.6, 36.1 and 26%, respectively (GOK, 2011). The prevalence of anaemia, iron deficiency and iron-deficiency anaemia in children ranges between 16.5–26.3, 9.4–21.8 and 4.9–13.3%, respectively (GOK, 2011). Zinc deficiency is among the most common causes of morbidity in developing countries. The prevalence of zinc deficiency in children aged 6 months to teenagers aged 14 years and expectant mothers ranges between 80.4–85.3 and 60.7–69.4%, respectively (GOK, 2011). In Kenya, expectant mothers and children are at risk of iron and zinc deficiencies due to consumption of mainly whole meal cereal-based food of low nutrient densities and high anti-nutrients such as phytates, tannins and oxalates (Veenemans, 2011).

In Kenya, there has been little attention given to seaweeds despite its commercial cultivation which began in 2010. Kenya has yet to find innovative ways to utilize the seaweed resources making it a potential gap that needs exploitation (Msuya *et al.*, 2014). Information on the chemical composition of seaweeds is key to their utilization. The Government of Kenya is making efforts to protect the natural resources base including the management of seaweed resource for commercial uses.

1.3 Justification

Seaweeds contain nutrients such as minerals, carbohydrates, protein, fibre and fat which have been incorporated in convenience foods such as bicuits, bread and buns. In Kenya, knowledge on the nutritional composition of seaweeds will help diversify the utilization of seaweeds in Kenya therefore opening the market for more seaweed-based products. Iron and zinc deficiencies are major public health concerns in Kenya contributing to the risk of morbidity and mortality among expectant mothers and children. Seaweeds from the Kenya coast may contain appreciable minerals such as iron and zinc which could be incorporated in foods to alleviate micronutrient deficiencies. The use of seaweeds as a mineral fortificant in convenience foods will revolutionize the micronutrient mitigation programs. Therefore, this study was aimed to explore the physical properties and sensory attributes of seaweed-based biscuits.

1.4 Hypotheses

i. There is no variation of the chemical composition of seaweeds by species, site and season.

ii. Seaweeds rich in iron and zinc and low in lead and cadmium cannot be utilized in the development of value-added biscuits.

1.5 Objectives

1.5.1 General objective

To evaluate selected Kenyan seaweed species for their nutritive potential in biscuits for improved health and nutrition among local communities in Kenya.

1.5.2 Specific objectives

1. To document the selected seaweeds collected from the study zone along the Kenya coast.
2. To determine the proximate composition and mineral content of seaweeds.
3. To evaluate the physical characteristics and sensory acceptability of the prepared biscuit.

CHAPTER TWO

LITERATURE REVIEW

2.1 Distribution, exploitation and cultivation of seaweeds

2.1.1 Classification and distribution of seaweeds

Seaweeds are photosynthetic, non-flowering, plant-like organisms that grow in the sea up to 180-meter depths, estuaries and backwaters on the hard substrates such as dead corals (Anantharaman *et al.*, 2013). They are classified based on morphology, pigmentation, chemical composition, anatomy, among other features as green algae (Chlorophyta), brown algae (Phaeophyta) and red algae (Rhodophyta) (Dawczynski *et al.*, 2007).

The seaweeds are distributed horizontally in different zonations namely supra-tidal (supra-littoral), intertidal (littoral) and subtidal (sub-littoral) regions of the seas and oceans (Stephenson and Stephenson, 1949). Green seaweeds are most commonly found in the intertidal zone characterized by shallow waters where sunlight is abundant. Common green seaweeds are species of *Ulva* (sea lettuce), *Enteromorpha* (green string lettuce), *Chaetomorpha*, *Codium* and *Caulerpa*. Brown seaweeds inhabit in the tidal zone characterized by coral reefs and deep waters. Common brown seaweeds are species of *Sargassum*, *Laminaria*, *Turbinaria* and *Dictyota*. Red seaweeds grow in subtidal waters commonly in tide pools attached to coral rocks. Common red seaweeds are species of *Gracilaria*, *Gelidiella*, *Eucheuma*, *Ceramium* and *Acanthophora* (Chapman and Chapman 1980).

2.1.2 Overview of development of seaweed exploitation

Human and seaweed interactions seem to date back to the Neolithic period (Dillehay *et al.*, 2008; Ainis *et al.*, 2014; Erlandson *et al.*, 2015), but the earliest written record of their human usage originates from China, about 1700 years ago (Yang *et al.*, 2017). For centuries, coastal populations harvested a wide variety of seaweeds from all algal groups. Initially, seaweeds were most often used for domestic purposes as food and feed, whereas later, industrial uses such as gels, fertilizers and medicine emerged (Delaney *et al.*, 2016). Early examples of utilization of seaweeds for medicinal purposes include the Chinese use of brown algae for goitre (16th century, Chinese herbal, ‘Pen Tsae Kan Mu’), *Gelidium* sp. for intestinal afflictions and dehydrated *Laminaria stipes* for the dilation of the cervix in difficult childbirths (Levine, 2016).

According to Food and Agriculture Organisation (FAO, 2016), of the top seven most cultivated seaweed taxa, three are used mainly for hydrocolloid extraction: *Eucheuma* spp. and *Kappaphycus alvarezii* for carrageenans, and *Gracilaria* spp. for agar; *Saccharina japonica* (formerly *Laminaria japonica*), *Undaria pinnatifida*, *Pyropia* spp. (formerly Porphyra) and *Sargassum fusiforme* are most important in human food usage. The main seaweed producing countries are China, Indonesia and the Philippines, which are also those that cultivated the greatest diversity of seaweed species (FAO, 2016).

Seaweed consumption in South-east Asia especially in China, Japana and Korea has been common and traditional, and has depended on taste and price (Chapman *et al.*, 2015; Gomez-Pinchetti and Martel-Quintana, 2016). In non-Asian, European and American markets, seaweed use as food has considered additional parameters such as nutritional value and ‘food for health’, with a strong consumer preference towards organic, sustainable and fair trade products (Chapman *et al.*, 2015; Gomez-Pinchetti and Martel-Quintana, 2016).

2.1.3 Current trends in seaweed cultivation

China and Indonesia are by far the largest seaweed producers with over 23 million tonnes of aggregated production in 2014. China produces mostly kelp for food that is *Saccharina japonica* and *Undaria pinnatifida*, and red algae belonging to the genera *Gracilaria* and *Poryphyra* (FAO, 2016). On the other hand, Indonesia produces mainly the carrageenophytes *Kappaphycus* and *Eucheuma* (FAO, 2016). Taken together, these leading five genera – *Saccharina*, *Undaria*, *Porphyra*, *Eucheuma*/*Kappaphycus* and *Gracilaria* – represent 98% of the world’s cultivated seaweed production (Suo and Wang, 1992; Pereira and Yarish, 2008). Furthermore, Chile, China, South Africa and Norway lead the exploitation of the wild stocks of seaweeds, of which kelps are the most sought after (FAO, 2016). In 2014, the leading seaweed farming countries, China and Indonesia, each produced more than 10 million tonnes, the Philippines and the Korean Republic over one million tonnes, whilst the Popular Democratic Republic of Korea, Japan, Malaysia and Zanzibar produced over 100,000 tonnes each. In the Americas, only Chile has appeared in the farming statistics tables, with 12,836 tonnes of cultivated *Gracilaria* spp. In Africa, Tanzania produce about seven tonnes of *Eucheuma denticulatum* and *Kappaphycus alvarezii* as the main cultivated species (Msuya *et al*., 2014; FAO, 2016). Cultivation of *Ulva* spp. has been set up in South Africa, based on an Integrated Multi-Trophic Aquaculture (IMTA) concept (Bolton *et al.,* 2009) and was adopted on a small scale in Israel (Shpigel, 2013; Neori, 2016). In Brazil, the cultivation of *Kappaphycus alvarezii* has been implemented during the last decade in coastal waters on the southern and south-eastern coasts for experimental purposes (Pellizzari and Reis, 2011).

In Eastern Africa, threats to the marine biodiversity are related to pollution, such as disposal of solid wastes or appearance of chemicals and metals around urban centres, coral mining, overfishing and uncontrolled projects that are developed in the areas, as well as climate change (Oyieke, 1996). Since seaweeds do not need any fertilization, but only rely on sun and water movement for their growths, the harvesting of them will not lead to an increased marine pollution. However, Eklöf *et al.* (2012) reported that algae cultivation can have a negative impact on the ecosystems in the surroundings such as limited resources of space, light and nutrients with other seaweed species. Biological diversity is essential to support planetary life systems and is linked to food security. Among the world’s plant species, 20% are marine, and the diversity of these species is decreasing with increasing depth and latitude. The most diversity is found in tropical coral reefs, seagrass beds and mangrove systems, and is well represented in East Africa (Oyieke, 1996), which indicates that there are still areas of improvement when it comes to algal cultivation.

In Zanzibar, the commercial algae cultivation is well-developed and goes back 30 years, to the late 1980's (de la Torre-Castro and Rönnbäck, 2004). When the commercial farming started on Zanzibar, the activities grew fast. Field studies show that possible reasons why the development was so rapid are related to the social and economic structure of Zanzibar. Some argue that the economic liberalisation of Zanzibar in the 1980's, where private companies had the possibility to export and import, played an important role in the rapid development (Pettersson-Löfquist, 1995) since almost all seaweeds are exported to other countries (de la Torre-Castro and Rönnbäck, 2004). Seaweed is seen as economically important in the Western Indian Ocean (WIO) region, and many view Kenya as a country with potential. Seaweed farming is recognized to be promising in generating better livelihoods for many people in the coastal communities of Kenya (Msuya *et al.*, 2014). Communities which depend on fishing, a sector with declining production, now have a new source of income. In the WIO region, a growing market is showing economical potential for cultivating seaweed (van Hoof and Steins, 2017). In the south coast of Kenya, the commercial farming of seaweeds started in 2010 (Wakibia, 2005; Wakibia *et al.* 2006; Wakibia *et al.*, 2011; Msuya *et al.*, 2014). Researchers now estimate Kenya to have a market capacity similar to that of Tanzania and promising opportunities to reduce poverty (van Hoof and Steins, 2017). However, the lack of a market is challenging for the seaweed farming in Kenya, and farmers have a need of government support to assist the commercialisation (Fredrick and Mutheu, 2018).

The Government of Kenya introduced a task-force on blue economy in 2017, with objectives to prioritize the use of sustainable marine resources. The country's interest is also reflected by the fact that Kenya in November 2018 hosted the first global conference with the theme, *Sustainable Blue Economy* (UNenvironment, n.y.). Infact, studies on seaweed in Kenya started in the 1960's and have increased since. By 1990, a survey on 15 sites along the Kenyan coast took place, together with studies on economic feasibility and socioeconomic aspects of the farming of *Eucheuma denticulatum* and *Kappaphycus alvarezii* (Bolton *et al.*, 2007). The results showed that the country has many suitable areas for farming along the coastline, with a possible opportunity to generate both direct and indirect employment. Pilot projects in Kenya have resulted in an employment of 100-200 seaweed farmers in Kenya by the year of 2017 (van Hoof and Steins, 2017). It has led to increased incomes, and have been shown to generate reduced fishing pressure. A place where seaweed farming is ongoing, is at Shimoni in Kibuyuni, Kwale county. The farming collective Kibuyuni Seaweed Farmers has increased its membership to 50 people by 2017. They now sell seaweed to The East African Seaweed Limited that buys and exports raw material to markets in South Africa and China (van Hoof and Steins, 2017).

2.2 Utilization of seaweeds

2.2.1 Seaweeds as human food

Among the chlorophytes, the common sources of food are species of *Monostroma, Caulerpa, Enteromorpha, Ulva* and *Codium*. In Japan, dried fronds of *Monostroma* are used to prepare “nori-jam” and soup. *Enteromorpha* and *Monostroma* are referred to as “aonori” (Japanese) (Ohno, 1993). In some Pacific regions, *Enteromorpha* is commonly known as “ele ele” (Hawaii), “lulua” (Fiji) and “nalumlum malekesa” (Vanuatu) is consumed raw, dried or cooked (Novaczek, 2001). Many Pacific islanders enjoy *Codium geppiorum* when paired with fish cooked in milk (Trono and Toma, 1993). *Caulerpa* spp. known as “green caviar”, “green sea feather” or “sea grapes” is commonly sold in many markets of the Pacific regions. *Caulerpa lentilifera* is prepared as a salad in the Indonesia and Philippines (Trono and Toma, 1993). *Caulerpa bikinensis, C. peltata* and *C. sertularioides* is a delicacy when paired with coconut milk (Payri *et al.*, 2000).

The phaeophytes such as *Laminaria* and *Undaria* are an edible and important resource in China, Japan and Indonesia (Tsutsui *et al.*, 2005). In South Vietnam, they are a delicacy consumed either boiled, raw or dried, with coconut milk, jelly, crushed ice, sweetened green beans (Tsutsui *et al.*, 2005). The seaweed *Cladosiphon okamuranus* is consumed as a salad in Japan (Thoma, 1997; Zhang *et al.*, 2004; Zhu *et al.*, 2009). The seaweed *Sargassum* also referred to as "horsetail" is dressed with soybean sauce or prepared as a soup in Korea (Madlener, 1977) and Hawaii (Novaczek, 2001). In the Pacific region, *Rosenvingea* or "slippery cushion", *Turbunaria* or "spiny leaf" are eaten as soup or omelette. *Colpomenia* or "papery sea bubble" is mostly consumed as a stew, chop soup or salad. *Hydroclatharus* or "sea colander", *Dictyota* or "brown", *Padina* or "sea fan ribbon weeds" is prepared as a soup, stew or food dressing (Novaczek, 2001).

The red seaweeds *Hypnea* or "maidenhair", *Acanthophora* or "spiny sea plant", *Laurencia* or "flower limu", *Asparagopsis* or "supreme", *Calophyllis* or "large wire weed", *Halymenia* or "red sea lettuce", *Scinaia* or "tender golden weed" are consumed when cooked, fresh (as pudding or salad), dehydrated (as spices) or used to make jellies, chopped or salted in the Pacific region (Novaczek, 2001). *Gracilaria* or "sea moss" is consumed fresh as a salad, a garnish for sashimi or as a homemade or commercial agar. (Madlener, 1977; Novaczek, 2001).

The *Gracilaria* species are consumed as fresh food in Hawaii. Commonly marketed seaweeds such as *G. parvispora*, *G. coronopifolia*, *G. tikvahiae* and *G. salicornia*. However, these seaweeds have a short post-harvest life of about four days (Paull and Chen, 2008). *Gelidiella* is consumed as a jelly in South Vietnam and Japan (Madlener, 1977; Novaczek, 2001; Tanaka and Nakamura, 2004). *Rhodymenia palmata*, *Alaria fistula*, *Chordaria flagelliformis* and *Porphyra umbilicalis* are mostly consumed in North America and Europe. *Porphyra* or "purple lever" is being consumed dried or fresh in Vietnam, China, Japan, Korea, Europe and North America (Madlener, 1977; Tanaka and Nakamura, 2004; Tsutsui *et al.*, 2005). Species of *Eucheuma* and *Kappaphycus* species are consumed when paired with sugar and coconut milk in South Vietnam and Indonesia (Tsutsui *et al.*, 2005).

Muraguri *et al.* (2016) attempted to incorporate the seaweed *Eucheuma denticulatum* in chicken sausages to improve on chemical and functional characteristics of the sausages with reduced cholesterol levels. From this study, incorporation of upto 1% seaweed content had improved chemical, functional and sensory characteristics with reduced cholesterol levels.

2.2.2 Seaweeds as thickening agents

Seaweeds are best known for their natural polysaccharides namely agar, carrageenan and alginates. These seaweeds gels are extensively used in various industries such as food, confectionery, textiles, pharmaceuticals, dairy and paper industries mostly as gelling, stabilizing and thickening agents (McHugh, 2003). Alginates are polysaccharides occurring as in cell walls of phaeophytes (Kalimuthu *et al.*, 1991). It is a linear 1,4-linked copolymer of β-D- manuronic acid (M) and α-L- guluronic acid (G) (Figure 2.1). They are essential compounds as thickening, gelling or stabilizing agents (Pérez *et al.*, 1992; McHugh, 2003).

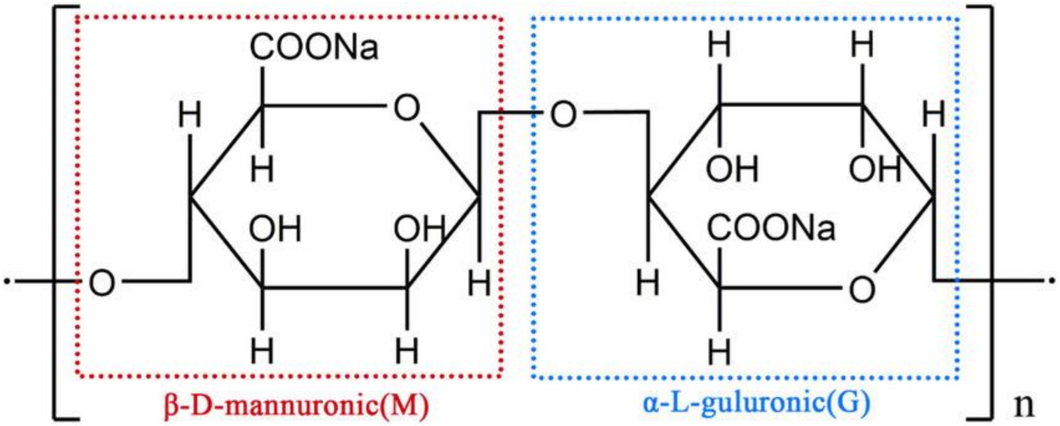


Figure 2.1: Structure of alginate – a linear 1,4-linked copolymer of β-D-manuronic acid (M) and α-L- guluronic acid (G).

Source: Xia *et al.*, 2019.

The phaeophytes such as *Ascophyllum nodosum*, *Macrocystis pyrifera*, *Laminaria* sp., *Sargassum* sp., *Turbinaria* sp. and *Padina* sp. are the major sources of extraction of alginates in Korea, China, Japan, South Africa, Namibia (Msuya, 2013).

Agars are mostly extracted from species of rhodophytes such as *Gelidium* and *Gracilaria* in China, Korea, Spain, Thailand and Japan. However, species of

Abnefeltia and *Pterocladia* have been used in Portugal, New Zealand, Russia and Japan (McHugh, 2003). It consists of chains of repeating alternate units of β-1,3-linked- D-galactose and a-1,4-linked 3,6-anhydro-L-galactose (Figure 2.2). Since the second world war, South Africa and Namibia have been successful in cultivating the species of *Gracilaria* for extraction of phycocolloids (McHugh, 2003; Msuya, 2013).

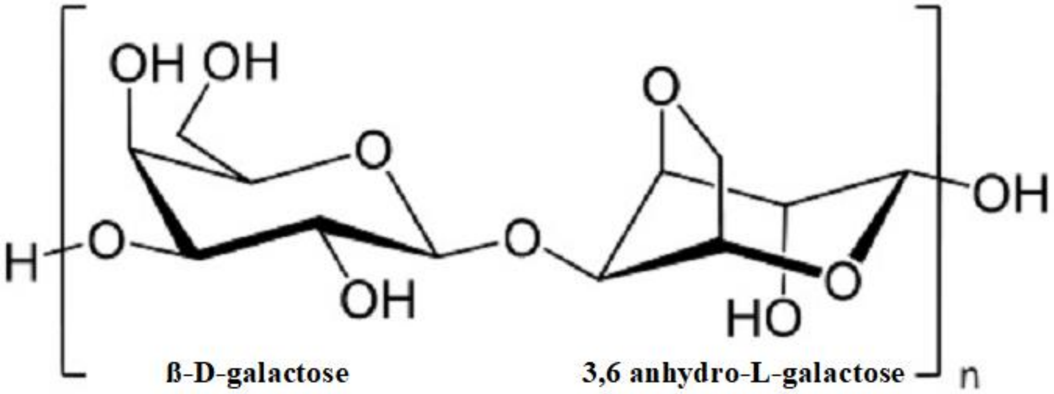
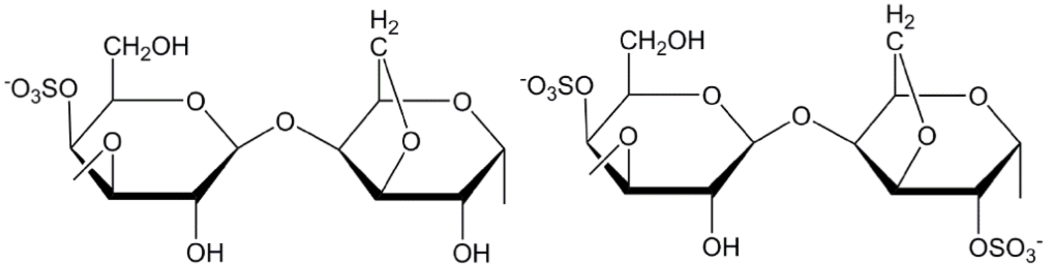


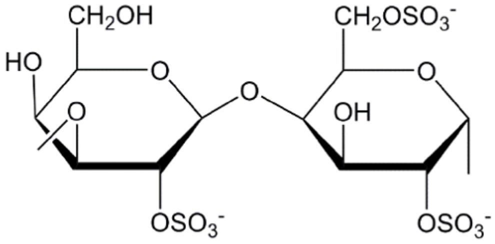
Figure 2.2: Structure of agar - β-1,3-linked copolymer of β-D-galactose and a-1,4-linked 3,6-anhydro-L-galactose.

Source: Siddhanta *et al*., 2015.

Wakibia (2005) and Wakibia *et al.* (2006) studied the growth rates of three commercial euchemoid isolates, originally from Philippines, *Eucheuma denticulatum*, and green and brown *Kappaphycus alvarezii* in three sites that is Gazi Bay, Mkwiro and Kibuyuni in the Southern coast of Kenya. They further characterized and compared carrageenan (Figure 2.3) extracts from the three isolates by studying yields, gel strength, viscosity and sulphate contents (Wakibia *et al*., 2006).



(A) kappa carrageenan (KC) (B) iota carrageenan (IC)



(C) lambda carrageenan (LC)

Figure 2.3: Structure of (A) kappa carrageenan, (B) iota carrageenan and (C) lambda carrageenan.

Source: Hassan *et al*., 2002.

Wakibia *et al.* (2011) compared two commercial euchemoids, the red *Eucheuma denticulatum* and the brown seaweed *Kappaphycus alvarezii* farmed in Gazi Bay and Kibuyuni sites in South coast and determined that the brown strain of *Kappaphycus alvarezii* was an economically viable venture for their phycocolloid in food, pharmaceutical and cosmetic industries.

2.2.3 Seaweeds in medicine industry

Seaweeds are rich in vitamins and bioactive substances. For example, the rhodophytes *Digenea* species produces kainic acid which is an effective vermifuge. *Sargassum* and *Laminaria* species has been used for cancer treatment in China. *Undaria* sp. has anti-viral properties which inhibit the *Herpes simplex* virus. Extracts of *Undaria* sp. are being used to treat HIV and breast cancers (Stein and Borden, 1984). The rhodophytes, *Ptilota* sp. produces lectin that preferentially agglutinates human B-types erythrocytes in vitro. The calcareous seaweed species of *Coralline* have been used in bone-replacement therapy (Stein and Borden, 1984; Khan and Satam, 2003). The extracts of the rhodophytes

Asparagopsis taxiformes and *Sarconema* sp. are used in management of goitre. The seaweed extract heparin is commonly used in cardiovascular surgery (Stein and Borden, 1984). The seaweed *Codium fragile* is known to have efficacy as a vermifuge for *Ascaris lumbriocoides*. Application of *Codium dichotomum* and *Grateloupia divaricate* have yielded positive results on primary school children in Hokkaido, Japan (Tokida, 1954).

The phaeophytes, *Fucales* spp. have antibacterial properties (Harlin, 1996; Hay, 1996) and as an inducible screen against UV radiation (Hay, 1996; Pavia *et al.*, 1997; Pavia and Brock, 2000). The seaweed, *Zonaria diesingiana* has been used in screening potential anticancer drugs (Fusetani, 1987). *Lobophora variegata* has bioactive compounds with potential anti-microbial defences (Kubanek *et al.*, 2003).

Some biologically active components in seaweeds such as alginic acid, peptides, carotenoids, phlorotannins and fucoidan have been shown to significantly prevent certain degenerative chronic diseases such as arthritis, inflammation, diabetes, cancer and hypertension. Therefore, seaweed-derived active compounds are a viable source of novel chemical agents for use as functional foods, cosmeceuticals and pharmaceuticals (Wijesinghe and Jeon, 2012).

Kaaria *et al.* (2015) reported that most isolates from the Kenyan seaweeds studied were bioactive against Gram negative bacteria compared to Gram positive bacteria and fungi. Also, activity against *Escherichia coli*, *Candida albicans* and *Staphylococcus aureus* was 47, 33 and 21%, respectively (Kaaria *et al.*, 2015). This indicates the presence of bioactive metabolites produced by the marine microbes. Studies by Kaaria *et al.* (2015) further indicated that the rhodophytes produced more bioactive isolates compared to the chlorophytes and phaeophytes probably due to differences in chemical composition and characterization of the algae. The above results reveal that Kenyan seaweeds have a potential antimicrobial activity.

2.2.4 Seaweeds as feed

Seaweeds were used as animal feeds in the first century BC by the Greeks. They were cheap sources of proteins, minerals and trace elements. The seaweed meals were given as supplements to cattle, pigs, poultry and other farm animals. Seaweed meals were obtained by grinding cleaned and washed seaweed species such as *Ulva, Padina, Enteromorpha, Dictyota, Gracilaria, Sargassum* and *Hypnea.* Seaweed meal was also mixed with fish meal and used as a poultry feed (Kaliaperumal, 2003). Farmers living near the sea in Norway used *Ascophyllum* spp. to make pig meal. *Rhodymenia palmata*, a rhodophyte, is referred to as cow weed in Britain and as horseweed in Norway. Dried seaweeds have been fed to farm animals in North America and Europe (Kaladharan, 2006).

Seaweeds are rich in protein (20–25%), carbohydrate (50–70%), vitamins, minerals and phytochemicals. When used in animal feed, cows produced more milk, chicken eggs became better pigmented and horses and pets became healthier (Kaladharan, 2006). Presence of Vitamin-E and tocopherols in seaweed increased the fertility rate and birth rate of farm animals. There was a substantial increase in milk production and milk fat content when seaweed meal was used as fodder. Feed supplemented with *Gracilaria* sp. to layer chicks improved the colour of egg yolks and increased egg size and production (Dave *et al.*, 1979). Dave *et al.* (1979) assessed the possibility of seaweeds as animal feeds in Japan, Germany, Britain and Norway. Cattle grazed on *Laminaria* sp. based diet had better natural resistance to common cattle diseases such as foot and mouth. In the USA, when hens were fed with an additional 1.25% seaweed to their daily ration, there was a reduction of the proportion of thin celled-eggs and when after 3 months the seaweeds diet was discontinued, the proportion of thin celled-eggs again increased (Dave *et al.*, 1979).

In Kenya, Arori *et al.* (2019) evaluated the potential of *Hypnea cornuta* and *Hypnea musciformis* from The Kenyan Coast as Nile tilapia (*Oreochromis niloticus*) fingerlings diets. Studies conducted showed significant growth performance and survival rate of the Nile tilapia fingerlings when fed with the rhodophytes *Hypnea cornuta* which contained less fibre content compared to *Hypnea musciformis*. Increased fibre content in fish feed reduces feed digestibility (Anderson *et al.*, 1984).

2.2.5 Seaweeds as organic fertilizer

The first record of the use of marine macroalgae as fertilizer was reported in Chinese and Greeks, where wet or dried seaweeds were scattered on the farms for providing nutrients. The species of phaeophytes such as *Ascophyllum*, *Ecklonia* and *Sargassum* were the most common as fertilizer and soil conditioners (McHugh, 2003). Over centuries, seaweeds have been partially substituted to manure in New Zealand, Australia, India, Great Britain, Japan, Spain, USA and France. An increase in yields of tomatoes, peaches, soybean, sweet potatoes, sweet corn and melons was observed when seaweed fertilizer was used (Chapman and Chapman, 1980).

Seaweeds contained minerals such as iron, copper, phosphorus, sulphur, magnesium, nitrogen, manganese, calcium, boron, potassium, and zinc which were found to increase sugar content in melons, and increased yields in root crops such as potatoes, and cabbages (Chapman and Chapman, 1980). The application of seaweed fertilizer was beneficial for plant growth due to the accelerated nutrient uptake which helped assimilate the carbohydrates and protein contents of the plants (Zia, 1990).

2.3 Chemical composition of seaweeds

2.3.1 Protein

Generally, phaeophytes such as *Himanthalia elongate*, *Ascophyllum nodosum*, *Fucus vesiculosus* and *Laminaria digitata* have the lowest protein content within the range of 5–15% of the dry weight (dw), whereas the chlorophytes and rhodophytes have the highest protein content ranging from 10–30% dw (Burtin, 2003). The red seaweed species of *Porphyra* and *Palmaria* can have higher protein contents ranging between 35–45% dw. These levels are comparable to those found in terrestrial vegetables such as soybeans. Burtin (2003) reported that the *Ulva* spp. had a protein content within the ranges of 15–20% dw which was higher compared to that found in *Ulva fasciata* (10.06% dw) reported by Muraguri *et al.* (2016).

The rhodophytes have phycobiliproteins such as phycoerythrin and phycocyanin (Boussiba and Richmond, 1979; Fan-jie *et al.*, 1984) which are made up of phycobilins (tetrapyrolic open core) linked to a proteic chain by a covalent bond. Recent studies indicate that phycobiliproteins possess antioxidant properties beneficial in the prevention and/or treatment of neurodegenerative diseases such as Alzheimer's and Parkinson's and chronic diseases such as cancers and cardiovascular diseases (Gonzalez *et al.*, 1999; Padula and Boiteux, 1999; Remirez *et al.*, 1999). The protein content of Kenyan seaweeds reported by Muraguri *et al.* (2016) ranged between 5.06-10.06% dw which were considerably lower than those reported by Burtin (2003).

2.3.2 Crude fat

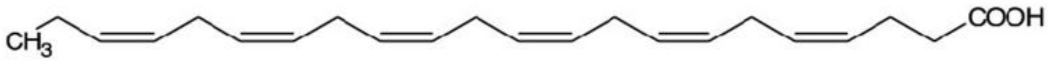
The crude fats represent only 1–5% of algal dry matter (Chan *et al.*, 1997; Burtin, 2003). Similar studies on the rhodophytes *Gracilaria* spp. by Benjama and Masniyom (2012) found a crude fat of 2.2–2.8% dw. Wong and Cheung (2000) obtained crude fat of <1.00% dw from rhodophytes *Hypnea* spp. studied in Hong Kong. Manivannan *et al.* (2009) reported that the crude fat content of phaeophytes *Sargassum wightii* and *Sargassum tennerimum* was 2.34 and 1.46%, respectively. Murugaiyan *et al.* (2012) found the highest lipid content in *Sargassum longifolium* (8.2 ± 1.57%) followed by *Turbinaria conoides* (3.0 ± 0.56%). Muraguri *et al.* (2016) reported crude fat levels of <1.78% dw from five seaweeds studied from the Kenya coast.

Le Tutour (1990) reported that algal lipids are primarily composed of polyunsaturated fatty acids particularly omega 3 (ω3) (Figure 2.4) and omega 6 (ω6) acids (Figure 2.5) which play a role in the levels of alpha-linolenic acid (ω3 C18:3) (Figure 2.4). On the contrary, the fatty acid profile of Kenyan seaweeds studied by Muraguri *et al.* (2016) were mainly composed of saturated fatty acids

(SFAs) ranging between 53.72-71.04%. Haugan and Liaaen-Jensen (1994) found that the red and brown seaweeds are rich in eicosapentaenoic acid (ω3 C20:5) and arachidonic acid (ω6 C20:4).

ALA: α-Linolenic acid C18:3 n-3

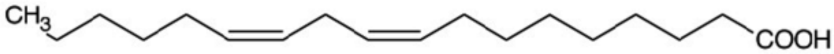
EPA: Eicosapentanoic acid C20:5 n-3



DHA: Docosahexanoic acid C22:6 n-3

Figure 2.4: Examples of Omega 3 fatty acids

Source: Mariamenatu and Abdu, 2021



LA: Linoleic acid C18:2 n-6

AA: Arachidonic acid C20:4 n-6

DPA: Docosapentanoic acid C22:5 n-6

Figure 2.5: Examples of Omega 6 fatty acids

Source: Mariamenatu and Abdu, 2021

The unsaponifiable fraction of seaweeds contain carotenoids such as lutein, β-carotene and violaxanthin in rhodophytes and chlorophytes, sterols and fucoxanthin in phaeophytes, terpenoids and tocopherols (Jensen, 1969; Piovetto and Peiffer, 1991; Haugan and Liaaen-Jensen, 1994). Extracts of the algal lipids of some edible seaweeds have shown antioxidant activity with tocopherol (Le Tutour, 1990).

2.3.3 Carbohydrates and dietary fibre

Seaweeds contain large amounts of carbohydrates ranging from 50 to 70% dw (Arasaki and Arasaki, 1983). This is comparable to carbohydrate contents reported by Muraguri *et al.* (2016) from Kenyan seaweeds. Other minor polysaccharides include ulvans (in chlorophytes), fucoidans (in phaeophytes), xylans (in certain rhodophytes and phaeophytes), floridean starch (in rhodophytes) and laminarin (in phaeophytes) (Lahaye and Thibault, 1990). Most of the algal polysaccharides such as fucoidans, carrageenans, agars and ulvans are indigestible in the human gut and can be regarded as dietary fibres (Lahaye and Thibault, 1990; Lahaye, 1991). Water-soluble and water-insoluble fibre fractions have been associated with different physiological effects. Many viscous soluble polysaccharides such as pectins and guar gum have hypoglycemic and hypocholesterolemic effects. Most water-insoluble polysaccharides such as cellulose are associated with a decrease in digestive tract transit time (Southgate, 1990).

Studies done on the polysaccharide, fucoidans showed that it had beneficial health properties such anti-coagulant, anti-thrombotic, anti-proliferative, anti-viral, anticancer, and anti-inflammatory applicable in therapeutic treatment (Angstwurm, 1995; Charreau, 1997). Some polysaccharides such as laminarans and xylans are rapidly and completely degraded by human intestinal bacteria whereas alginates are only partly degraded resulting in substantial production of short-chain fatty acids (Burtin, 2003) beneficial to human health.

2.3.4 Minerals

Seaweeds are known to draw an incomparable composition of minerals and heavy metals from the sea (MacArtain *et al.*, 2007). Some seaweeds contain ash contents of up to 36% dw (Ruperez, 2002). Compared to terrestrial plants and animal products, seaweeds contain higher mineral content (Ruperez, 2002; MacArtain *et al.*, 2007). Muraguri *et al.* (2016) reported mineral content ranging from 12.79 to 27.13% dw from the five Kenyan seaweeds studied. Calcium content in seaweeds accounts for about 7% of the dry algal weight (Garrow *et al.*, 1997; Teas *et al.*, 2004). Seaweed consumption may be useful in the case of children, adolescents, expectant mothers and the elderly who are exposed to the risk of calcium deficiency.

Seaweeds are sources of trace elements such as iron and zinc which are essential for maintenance of human health. Matanjun *et al.* (2009) reported the iron content of 68.21 mg/100g dw in *Sargassum polycystum* from Malaysia. Vizetto-Duarte *et al.* (2016) recorded iron content ranging 17-50.81 mg/100g dw from *Cystoseira* spp. from Portugal. A study by Rohani-Ghadikolaei et al. (2012) reported iron contents of 46.4, 58.9 and 85 mg/100g dw from *Ulva lactuca*, *Sargassum ilicifolium* and *Gracilaria corticata*, respectively from the Persian Gulf of Iran. The iron contents of Kenyan seaweeds *Hypnea musciformis*, *Sargassum oligocystum*, *Ulva facsciata*, *Eucheuma denticulatum* and *Laurencia intermedia* were 7.34, 25.82, 51.39, 48.80 and 30.71 mg/100g dw, respectively (Muraguri *et al*. 2016). Vizetto-Duarte *et al.* (2016) reported the zinc content of 10.59, 11.38 and 10.67 mg/100g in *Cystoseira tamariscifolia*, *Cystoseira nodicaulis* and *Cystoseira baccata*, respectively. Iron and zinc are trace elements of public health concern globally. The elements Iron and zinc are abundant in phaeophytes and rhodophytes (Garrow *et al.*, 1997; Ruperez, 2002; Burtin, 2003; Teas *et al.*, 2004; Wada *et al.*, 2011; Benjama and Masniyom, 2012) thus could be utilized to alleviate iron and zinc deficiencies among children and expectant mothers.

2.3.5 Effect of monsoon seasons on chemical composition of seaweeds

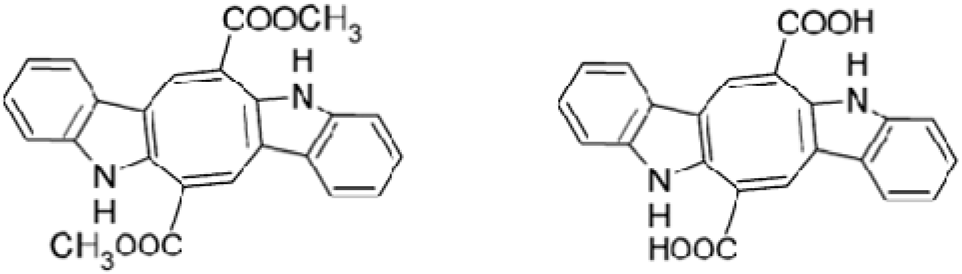
Seaweeds are exposed to seasonal variations of abiotic factors that influence metabolic functions such as photosysnthetic activity and growth rates; and levels of chemical constituents (Orduna-Rojas *et al.*, 2002). Seasonal variations in the chemical composition and nutritive value of seaweeds have been reported in seaweeds from Hong Kong (Wong and Cheung, 2000), Egypt (Khairy and El-Shafay, 2013), Brazil (Marinho-Soriano *et al.*, 2006), India (Banerjee *et al.*, 2009) and Malaysia (Aroyehun *et al.*, 2019). Aroyehun *et al.* (2019) reported that carbohydrates, protein and dietary fibre of seaweeds were significantly higher (p<0.05) in Northeast Monsoon (NEM) than in Southwest Monsoon (SWM). There were no significant differences in crude lipid and moisture contents of the studied seaweed *Gracilaria manilaensis* (Aroyehun *et al.*, 2019). The Malaysian climate is modulated by the Southeast Asian Monsoon with a cycle of two opposite regimes namely, NEM and SWM. The SWM begins in late May and ends in late September, while the NEM commences around November and ends in March of the subsequent year (Phang *et al.*, 2019). Malaysia receives substantial rainfall all year-round, but the amount peaks during the NEM period (Juneng and Tangang, 2008). The SWM is characterized by less rainfall of about 4.8 mm. According to available reports, the average annual total rainfall and evaporation were 1862 mm and 1098 mm, respectively (Awang *et al*., 2015). However, the rate of evaporation is affected by temperature and cloudiness, and when it is cloudy, there is less sunshine, resulting in less solar radiation and lower temperature. The drier months have higher evaporation, while the rainy months have lower, influencing water body salinity (Baharim *et al.*, 2016; Malaysia, 2009; Wong *et al.*, 2016).

Banerjee *et al.* (2009) observed significant variations in carbohydrates and protein in the seaweed *Catanella repens* in the pre-monsoon, monsoon and post monsoon periods. Carbohydrate content was highest in pre-monsoon and lowest in post monsoon period. From the studies, protein and carbohydrates were inversely correlated. Higher protein content and lower carbohydrate content were recorded during monsoon period. This period was characterized by high nitrate load in the ambient waters and low surface water temperatures. The inverse relationship between carbohydrates and protein with temperature and salinity has been pointed out by Mourandi-Givernaud *et al.*, (1993). The trend may be attributed to the positive role of light intensity, temperature and decrease in nitrogen for carbohydrate synthesis, while for the protein these parameters act inversely (Rosemberg and Ramus, 1982; Rotem *et al.*, 1986).

Most studies have reported that variation of chemical composition of seaweeds is probably due to changes in seasons (Mabeau and Florence, 1993). The East African region is influenced by the Northeast and South east Monson cycles (Yarish and Wamukoya, 1990). However, there is limited knowledge on the effect of seasonal oscillation on the chemical properties of Kenyan seaweeds.

2.4 Potential hazards associated with consumption of seaweeds

Some seaweeds contain toxins that have adverse effects on humans and to their own ecosystems. Toxins from a *Caulerpa* sp. namely, caulerpin and caulerpinic acid (Figure 2.6), have insecticidal activity against common mosquito, *Culex pipiens* (Alarif *et al.*, 2010).



(A) Caulerpin

(B) Caulerpinic acid

Figure 2.6: Structure of (A) Caulerpin and (B) Caulerpinic acid.
Source: Rushdi *et al*., 2020.

Several studies by, Gregson *et al.* (1979), Fusetani and Hashimoto (1984) and Noguchi *et al.*, (1994) indicated that food poisoning by *Gracilaria* species caused physiological activities in humans such as hypertension, bleeding, nausea and diarrhoea. However, there is limited evidence on the number of reports of, and studies dealing with, illness-causing toxins in seaweeds (Botana, 2007; Kilinç *et al.,* 2011).

The consumption of seaweeds has been associated with concerns over ingestion of contaminants and heavy metals such as cadmium and lead (Brandon *et al.*, 2014). Heavy metals are capable of causing adverse effects to human health if present in foods above the permissible limits set by Codex Alimentarius Commission (CAC, 2003). They have been given special attention in many countries throughout the world due to their toxicity effects when accumulation exceeds the permissible limits (Das, 1990; CAC, 2003; Jarup, 2003).

There is no known function of lead in the human body. Lead toxicity has adverse effects on every organ of the human body. Lead has the ability to mimic calcium thus affecting the actions of calcium-dependent processes. It binds to proteins via sulfhydryl, amine, phosphate and carboxyl groups. Lead primarily affects the metabolism of vitamins D, renal functions, blood cells, peripheral and central nervous systems. It is associated with neurological disorders, reproductive toxicity, developmental effects and hypertension (WHO, 1995; Jaishankar *et al.*, 2014). Cadmium has no useful biological functions. It interferes with some zinc-dependent functions, thereby inhibiting nutrient utilization and various enzyme reactions. It is involved in the generation of free-radical tissue damage by catalysing oxidation reaction (Schuhmacher *et al.*, 1993; WHO, 2001; Jaishankar *et al.*, 2014).

2.5 Micronutrient malnutrition

Micronutrients are a non-energy yielding group of nutrients comprising of minerals and vitamins consumed in very small dosages daily for optimal health and well-being (Ramakrishnan, 2002). They are not synthesized by the body thus should be availed through diet (Rolfes *et al.*, 2008). Micronutrients play important biological functions such as immune modulation, cellular proliferation, gene expression, regulation of enzymes and hormone actions, differentiation, growth and development. They are involved in metabolic processes and utilization of the macronutrients (WHO, 2001; Prasad, 2011).

Micronutrient malnutrition (MNM) is a global concern resulting in huge costs on societies in terms of poor quality of life, reduced economic productivity, morbidity and mortality (WHO, 2001). Micronutrient deficiencies result from poor dietary quality, intake of inadequate food, poor nutrient bioavailability attributed to the presence of anti-nutrients and mode of food preparation and/or the presence of other infections (WHO, 2001; Ramakrishnan, 2002).

2.6 Role of iron and zinc in human nutrition

2.6.1 Iron deficiency

Iron is present as haemoglobin in the erythrocytes of the human body. The main function of haemoglobin is to transport oxygen from the lungs to other body tissues (Brabin *et al.*, 2001a). Iron is essential in several enzyme systems involved in oxidative metabolism. In the liver, iron is stored as haemosiderin and as ferritin (Mason *et al.*, 2001; WHO, 2001).

Iron deficiency is the most widespread dietary disorder (Mason *et al.*, 2001). The main prevalence is among the vulnerable population groups such as expectant mothers (65%), 50% of infants and children aged 1–2 years, 25% of preschool-aged children, 40% of school children, 30–55% of adolescents, 35% of non-expectant mothers. Notably, iron deficiency and iron-deficiency anaemia are more prevalent in sub-Saharan Africa and in Asia, and where close to 90% of expectant women become anaemic (Veenemans, 2011; Brabin *et al.*, 2001a; Unnevehr *et al.*, 2007).

Iron-deficiency anaemia (IDA) contributes significantly to high levels of neonatal and maternal mortalities among the poor and vulnerable populations. The ‘hidden’ impact of iron deficiency include impaired cognitive function and physical performance, and increased child and maternal mortality (Brabin *et al.*, 2001b; Mason *et al.*, 2001; WHO, 2001; Ramakrishnan, 2002; Unnevehr *et al.*, 2007).

According to the National survey report (GOK, 1999), the lake basin and coastal and northern semi-arid lowlands lead in regards to distribution of the burden of anaemia per unit of surveyed population. The prevalence of IDA among adolescent schoolgirls in Western Kenya was found to be ranging from 19.8 to 30.4% (Leenstra *et al.*, 2004). According to United Nations Children's Fund (UNICEF, 2009) the prevalence of Iron deficiency anaemia (IDA) among children under five years was 34.5% based on anaemia prevalence of 69%. Foote *et al.* (2013) reported the prevalence of IDA among children aged 6-35 months in Nyando, Kisumu County was 61.9%. Another study by Kenya Demographic Health Survey (KDHS, 2008) reported that 71.8% of children in Central Province of Kenya do not consume iron-rich foods which is probably the reason for the high prevalence level of IDA among children.

2.6.2 Zinc deficiency

Zinc is a trace mineral involved in cellular growth, especially in the production of enzymes necessary for the synthesis of ribo-nucleic and deoxyribo-nucleic acids (RNA and DNA) (Pfeiffer and Braverman, 1997; Sandstead, 1997). In the brain, zinc binds to proteins, thus contributing to its structure and function (Hesse, 1979; Friel *et al.*, 1993). Zinc is critical in wound healing, body tissue growth, bone mineralization, taste acuity, growth and maintenance of connective tissue, thyroid function, immune system function, blood clotting, prostaglandin production, cognitive functions, foetal growth and sperm production (Friel *et al.*, 1993; Pfeiffer and Braverman, 1997). In expectant mothers, the lack of zinc may result in a decrease in foetal brain cells hence affecting their mental development. Among children, inadequate zinc hinders their normal development and growth, detrimental to intellectual development and reproductive system health. Zinc-deficient adult males may suffer from prostatic hyperplasia, reducing the reproductive function of the system and affect fertility (Prasad, 1991; Friel *et al.*, 1993).

It is often difficult to identify zinc deficiency due to its non-specific clinical manifestations characterized by a wide range of symptoms without suitable biomarkers of zinc deficiency (Sandstead, 1997). In children, stunting is among the possible consequences of zinc deficiency but not limited to low blood pressure, retarded bones, loss of appetite, loss of sense of smell and taste, weight loss, pale skin, hair loss, fatigue and white spots under fingernails (Hotz and Brown, 2004).

According to the national survey report (GOK, 1999), high risk of hypozincaemia is likely to occur in about half of the population in Kenya. According to the national survey (GOK, 1999) conducted among 541 children revealed a risk of 50% to zinc deficiency based on proportion of low serum zinc of 50.8%. Another study of 555 rural children in Embu district showed low baseline serum zinc concentration in 65.6% of the children (Siekmann *et al.*, 2003). A high prevalence of low plasma zinc is a reasonable indicator of zinc deficiency among children. Among children, anaemia, hookworm and diarrhoea were the main risk factors for hypozincaemia (GOK, 1999). Among mothers, anaemia, hookworm, respiratory illnesses and maternal nutrition were significant factors for hypozincaemia. (GOK, 1999). Pregnancy significantly increased the risk of zinc deficiency (GOK, 1999). In the case of men, Schistosoma infections and Vitamin A deficiency were each associated with increase in the risk of hypozincaemia (GOK, 1999).

2.6.3 Mitigating iron and zinc deficiencies

The widespread recognition of the importance of minerals and vitamins to human health, and the potential to address their deficiencies relatively cheaply through supplementation and fortification has led to several efforts to support traditional interventions (Caballero *et al.*, 2005; Unnevehr *et al.*, 2007; Uchendu, 2011). Some of the traditional interventions included consumption of whole grain cereals and consumption of indigenous vegetables (Caballero *et al.*, 2005). Fortification and supplementation are often aimed to address the symptoms of micronutrient deficiencies (Varea *et al.*, 2011). Among the strategies recommended in the National survey report (GOK, 1999) to address challenges associated with micronutrient malnutrition particularly iron and zinc deficiencies include nationwide efforts towards increasing micronutrient density through, among others increasing bioavailability and food fortification of commonly consumed foodstuffs and condiments.

Supplementation involves providing substantially high doses of micronutrients often in the form of capsules, pills or syrups rather than in food. Its main advantage is the capability of supplying optimal amounts of specific nutrients such as fat-soluble vitamins in a highly absorbable form. It is the most appropriate for targeted populations with a moderate to high prevalence of the deficiency such as in an acute food shortage and/or during pregnancy (Untoro *et al.*, 2002; Ottaway, 2008). Global patterns reveal trends towards increased usage of dietary supplements in healthy populations in both developed and developing countries. However, there is limited information on prevalence of dietary supplement use and dietary practices in developing countries in the Sub-Saharan Africa. In Kenya, an uptake of 43.5% among gym users (Wachira, 2011) and 15.5% among Kenya league rugby players (Kimiywe and Simiyu, 2008) has been reported. A survey conducted by Gikwa (2019) among secondary school teachers in Kikuyu Sub-County, Kiambu County revealed that the prevalence of dietary supplements among the teachers was 28.7%. The main types of supplements taken included omega 3 and 6 class of oils which were used by 60.8% of users followed by calcium supplements (56.9%). The majority (59.6%) of the participants took dietary supplements on prescription, followed by 29.8 and 25.5% who took them to prevent diseases or deficiencies and promote good health, respectively, while 2.1% used them to prevent aging or for cosmetic purposes.

Food fortification involves the addition of micronutrients to processed food products. This approach is associated with rapid improvements in the micronutrient status of the target population group at an affordable cost (Mannar and Sankar, 2004). This is the most commonly used public health intervention method (Mannar and Sankar, 2004; Meenakshi *et al.*, 2010). In Kenya, the Ministry of Health identified food fortification as a High Impact Nutrition Interventions (HiNi) to reduce micronutrient malnutrition (WHO, 2016). Essential micronutrients such as folate, iron, zinc, vitamins A and some group B vitamins are added to food in order to improve its nutritional quality, to fulfil the role of other foods in the human diet and the provision of a public health benefit with the least risk to health (Ottaway, 2008; WHO, 2016).

Fortification is done in three ways (Ottaway, 2008; WHO, 2016). First, by restoring the nutrients lost during food processing to their natural level (for example restoring B-vitamins which are lost during milling of cereals). Second, by increasing the level of a nutrient above that normally found in the food (for example adding extra iron and zinc to milled cereals). Third, by adding nutrients that are not normally present in a food item otherwise considered a good vehicle for delivering micronutrients to the consumer (for example putting vitamin A into sugar, or iodine into salt) (Ottaway, 2008; Meenakshi *et al.*, 2010). Lessons learnt from salt iodization programme in Kenya should be utilized (GOK, 1999). Food vehicles such as salt, milled cereals, milk, margarine, oils and fats, infant formulas, sugar and biscuits (referred as cookies, in some continents) are widely used in fortification (Tulchinsky, 2010).

2.7 Baking biscuits

2.7.1 Biscuits in general

Biscuits are the most popular and versatile food products (Nagi *et al.*, 2012) appreciated for their convenience, conservation, texture, varied taste, appearance at an affordable cost. They are basically prepared with soft (refined) wheat flour, sugar, salt, shortening and water (Jan *et al.*, 2016). The main sequential operations in the production of biscuits are ingredient metering, dough mixing, dough sheeting, dough sheet relaxation, biscuit shape forming, biscuit baking, cooling and packaging (Chevallier *et al.*, 2000).

During baking, the most apparent interactions are volume expansion, enzymatic activities, crust formation, protein coagulation and partial gelatinization of starch in the batter (Mondal and Datta, 2008). During baking, the biscuit batter undergoes complex biochemical and physicochemical reactions. These reactions involve fat-melting, loss of starch granular structure, protein denaturation, Maillard and browning reactions and batter expansion. resulted from water evaporation as well as production and thermal expansion of gases (Chevallier *et al.*, 2002). Ultimately, these reactions affect the sensory acceptability of biscuits. Other factors such as the quantity of the ingredients, type of the flour and the protein content of wheat flour influence the physical properties of biscuits (Pyler, 1982; Gaines, 1993).

2.7.2 Biscuits from unconventional ingredients

Many authors have incorporated protein-rich, mineral-rich or fibre-rich substitutes in biscuits to make them functional foods (Ranjana *et al.*, 1998; Ovando-Martinez *et al.*, 2009; Chauhan *et al.*, 2016). These substitutes include composite flours from terrestrial crops such as amaranth, banana, maize, pigeon peas, soybean, sweet potatoes (Ranjana *et al.*, 1998; Ovando-Martinez *et al.*, 2009; Chinma *et al.*, 2011; Silky and Tiwari, 2014; Chauhan *et al.*, 2016; Adeola and Ohizua, 2018) and seaweeds (Kumar *et al*., 2018).

Ovando-Martinez *et al.* (2009) used banana flour in biscuits due to its indigestible carbohydrates, minerals, and antioxidant activity. Silky and Tiwari (2014) prepared biscuits using pigeon pea flour. Some gluten-free flours such as sweet potato flour were not only used in dough conditioning but also improving the toasting properties and nutritional composition of biscuits and bread (Adeyeye and Akingbala, 2015). Kumar *et al*. (2018) utilized *Caulerpa racemosa*, one of the most predominant seaweeds along the Indian coastline with immense nutritional potential (Matanjun *et al.*, 2008), in formulation of functional biscuits. The study revealed that biscuits with more than 5% seaweed had decreasing sensory desirability in terms of flavour, colour, appearance, texture and overall acceptability (Kumar *et al*., 2018).

2.7.3 Role of ingredients in biscuit baking

Baked products differ from other food products due to the usage of leaveners to yield low density baked products. Leavening is done by CO_2 produced from yeast fermentation or baking soda (Rao and Bhargavi, 2017). Leavening is produced only if the gas trapped in a system that will hold it and expand along with it. Therefore, much of baking technology is the engineering of food structures through the formation of correct dough and batter in order to trap leavening gases by the application of heat (Balaji, 1991; Rao and Bhargavi, 2017).

Addition of sugar decreases relaxation time and dough viscosity. It promotes an increase in length and reduction in the weight and thickness of the biscuit. Sugar attributed to the crunchiness and highly cohesive structure of biscuits (Baltasvias *et al.*, 1999; Rao and Bhargavi, 2017). Addition of fat softens the dough and causes a reduction in viscosity and relaxation time resulting in a biscuit with a friable structure. Water content increases the intake of total specific energy (TSE) resulting in a reduction of dough viscosity and relaxation time. The biscuits become longer, with slightly smaller thicknesses (Rao and Bhargavi, 2017).

Wheat flour with about 14-20% protein content promotes an increase in the water absorption of the flour, and in the viscosity and relaxation time of the dough (Rao and Bhargavi, 2017). Fustier *et al.* (2009), studied the substitution of commercial soft wheat flour with starch tailing fractions isolated from wheat flour on dough rheology and characteristics of semi-sweet biscuits. Increasing the starch tailing fractions influenced the consistency and hardness of the dough, whereas lowered crunchiness of the biscuits due to higher pentosan (Fustier *et al.*, 2009).

2.7.4 Physical and sensory characteristics of biscuits

The physical properties of biscuits made from different composition and formulations have always been determined in three-point bending test (Baltasvias *et al.*, 1999). Commonly, a reduction of fat increases the fracture stress and the absolute value of biscuits. The use of sucrose syrup results in an enhanced brittleness of biscuits, compared with crystalline sucrose. Substitution of part of the wheat flour for starch does not substantially affect the physical properties of the biscuits. Sugar-free biscuits have a substantially lower fracture stress (Baltasvias *et al.*, 1999; Cronin and Preis, 2000).

Cronin and Preis (2000) studied the physical properties of biscuits during baking based on the dispersion of weight and thickness of biscuits. In this study, the dispersion in biscuit weight was mainly due to variation of the dough piece weight and was linked to longitudinal variation of the thickness of the dough sheet where the dough pieces were cut.

2.8 Seaweed resources in Kenya

Most of the initial studies done on the seaweeds of Kenya have mostly been taxonomic in nature (Moorjani, 1977). In comparison to other Indian Ocean countries, the seaweeds of Kenya are relatively well documented (Coppejans *et al.*, 2001; Bolton *et al.*, 2003). A comprehensive list of seaweeds recorded includes a total of 386 species (214 rhodophytes, 116 chlorophytes and 56 phaeophytes), plus an additional 19 species of Cyanophyta (Silva *et al.*, 1996; Bolton *et al.*, 2007).

Currently, studies on Kenyan seaweeds indicate they are potentially rich in nutrients (Mwalugha *et al.*, 2015; Muraguri *et al.*, 2016). Further, seaweeds of Kenya have been evaluated for their economic potential as sources of agar and carrageenan (Yarish and Wamukoya, 1990; Wakibia, 2005; Wakibia *et al.*, 2006; Wakibia *et al.*, 2011) and in the food industry (Muraguri *et al.*, 2016). Nevertheless, innovative ways to utilize the seaweed resource remains to be a potential gap that needs exploitation (Msuya *et al.*, 2014).

Seaweed farming has been identified as a good socio-economic activity in the coastal areas (Msuya, 2006). It is aimed at diversifying livelihood opportunities for poor fishing communities whose livelihoods have been put at serious risk by diminished capture fisheries (Msuya *et al.*, 2014). In Kenya, seaweed farming is being practiced in the south coast at Kibuyuni, Nyumba Sita, Mkwiro, Funzi and Gazi Bay. The main species cultured are *Eucheuma denticulatum* referred as ‘spinosum’ and *Kappaphycus alvarezii* known as ‘cottonii’ (Msuya *et al.*, 2014).

2.9 Conclusions from literature review

There is limited information on nutritional and mineral composition of Kenyan seaweeds. Out of the 386 species of seaweeds documented from the Kenya coast (Bolton *et al.*, 2007), Muraguri *et al.* (2016) reported the chemical composition of only five seaweeds namely; *Eucheuma denticulatum*, *Hypnea musciformis*, *Laurencia intermedia*, *Sargassum oligocystum* and *Ulva fasciata*. Arori *et al*. (2019) reported chemical composition of *Hypnea cornuta* and *H. musciformis*.

Further, the effect of Monsoon cycles (NEM and SEM) on the chemical composition of saweeds from the Kenya coast is not well studied. Arori *et al*. (2019) reported variation of chemical composition of *Hypnea cornuta* and *H. musciformis* collected from Mkomani and Mtwapa in the months of October (SEM), November and December (NEM) in 2017. The study was limited to rhodophytes. The green and brown seaweeds were not studied. In addition, knowledge on variation of nutritional composition of seaweeds with sites and months is important to determine suitable sites and periods for seaweed collection and potential farming patterns for better seaweed utilization. Studies on application of Kenyan seaweeds in food industry is limited to the extraction of food thickening gels from the seaweeds *Eucheuma denticulatum* and *Kappaphycus alvazerii* (Wakibia, 2005; Wakibia *et al.*, 2006; Wakibia *et al.*, 2011) and utilization of the red seaweed *Eucheuma denticulatum* in chicken sausage making (Muraguri *et al.*, (2016). There is need to study more seaweeds for better utilization in food product development. From the above gaps, this study aimed to collect seaweeds along the Kenya coast, determine their chemical composition for potential utilization in biscuits for improved health and nutrition.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Sampling sites

The sampling sites were three (3): Mkomani (located at latitude 4º 3’ 25.26” S, longitude 39º 41’ 3.50” E), Kibuyuni (located at latitude 4º 38’ 53.11” S, longitude 39º 19’ 40.21” E) and Mtwapa (latitude 3º 56’ 40.51” S, longitude 39º 46” 22.19” E) at the Kenya coast (Figure 3.1). The sites were selected because they had a wide diversity of seaweed species (Bolton *et al.*, 2007).

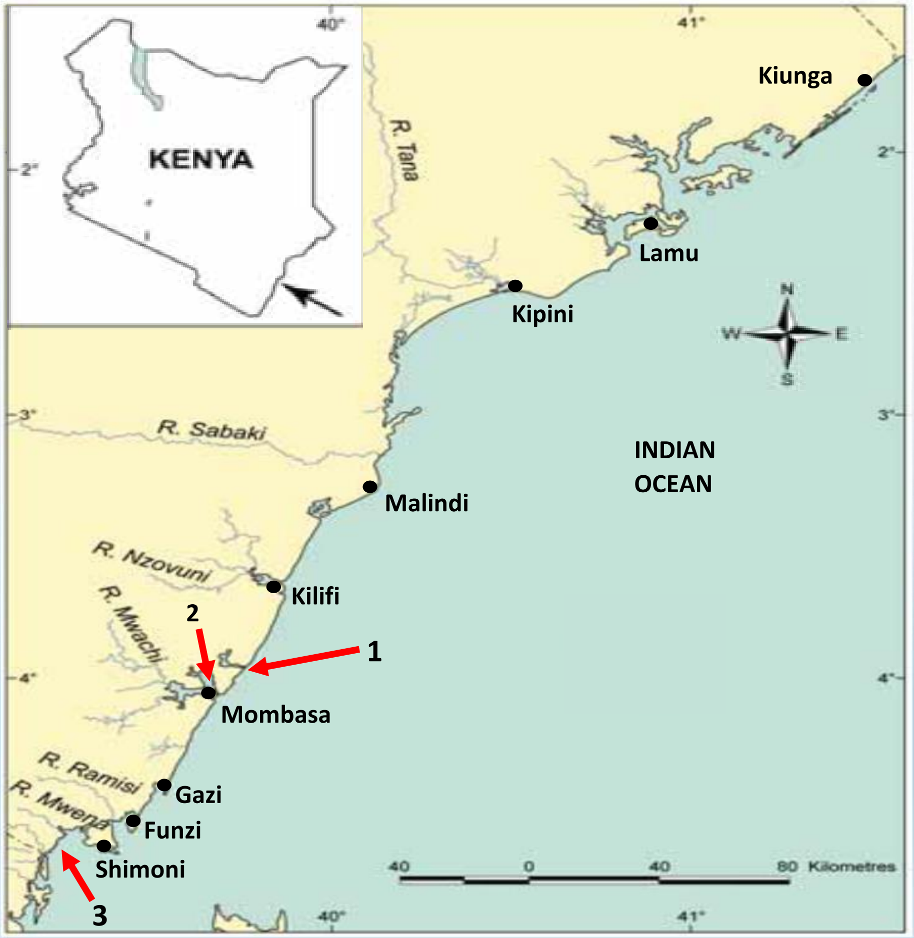


Figure 3.1: Map of Kenya showing the seaweed sampling sites (1 – Mtwapa, 2 – Mkomani and 3 – Kibuyuni).

3.2 Research design

3.2.1 Source of samples

The study adopted a complete randomized design for the collection of selected seaweed species from three different sites; Mkomani and Kibuyuni in March, July and October in 2013 and in Mtwapa in July and October 2013. A purposeful sampling technique was used to collect selected seaweed species of potential nutritional potential from the different sites. The chemical analyses were conducted in triplicates.

3.2.2 Formulation of biscuits

A complete randomized design was employed in this stage where the baking flour was substituted with the selected seaweed upto 5% by creating six treatments (0, 1, 2, 3, 4 and 5%). The physical properties of the biscuits were determined by evaluating the thickness, width and spread factor of the baked biscuits in triplicates.

3.2.3 Sensory evaluation

This study employed a complete randomized design for evaluation of sensory attributes of the prepared biscuits. The seaweed biscuits were presented to 28 untrained panelists comprising of staff and students of JKUAT. A simple random sampling technique was used to select panelists from the population in JKUAT. The panelists used a questionnaire (See Appendix 1) to evaluate the seaweed biscuits in 5 defined criteria: colour, hardness, aroma, taste and overall consumer preference.

3.3 Collection, identification and preparation of algal samples

3.3.1 Collection of seaweed samples

The samples of selected seaweeds species were collected in Mkomani and Kibuyuni in March, July and October in 2013 and in Mtwapa in July and October 2013 so as to cover both the Northeast Monsoon (NEM) and Southeast Monsoon (SEM) seasons in order to determine variation in their chemical composition.

Collection of seaweeds samples was done in the daytime at low tide (Plate 3.1). Seaweed samples were picked by hand and immediately washed with seawater to remove foreign particles, sand particles and epiphytes. About five kg fresh weight of seaweed samples were kept in clean buckets and immediately transported to biological laboratories in Kenya Marine and Fisheries Research Institute (KMFRI), Mombasa, for identification and sun-drying.

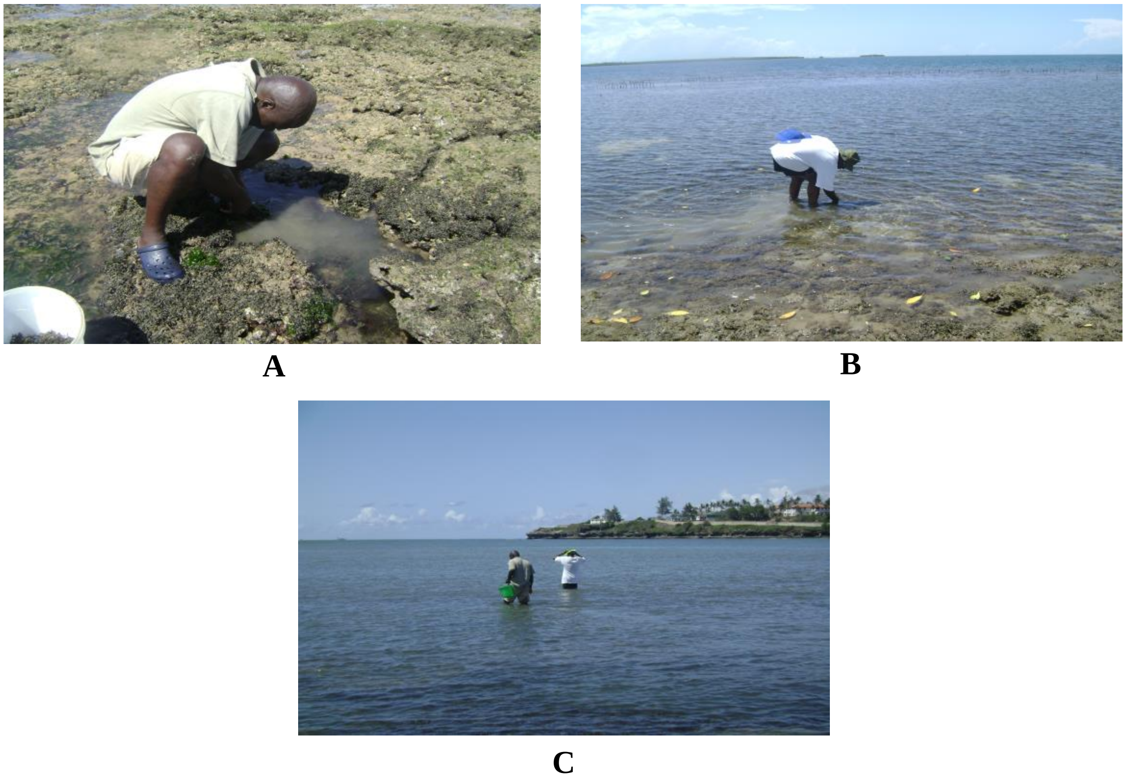


Plate 3.1: Collection of seaweed species at (A) Mtwapa, (B) Kibuyuni and (C) Mkomani.

3.3.2 Identification of selected seaweeds

The identification of selected seaweed collected was guided by Prof. Joseph G. Wakibia, Marine Botanist from Botany department, JKUAT. This involved carefully studying the morphological differences between the genera/species and taxonomic characteristics. The taxonomic description of the species and anatomical characteristic of the species were referred from documented reference guidelines (Trono, 1999). The seaweed species were compared to the reserved voucher specimens in KMFRI.

3.3.3 Preparation of algal samples

The identified seaweed samples were sundried and transported to the Food Biochemistry Laboratory, Department of Food Science in Jomo Kenyatta University of Agriculture and Technology (JKUAT). They were washed with tap water to remove adhering salts and any foreign matter. A final rinse was done using plenty of deionized water. Seaweeds were spread on blotting paper to drain off excess water before they were sundried to constant weight; ground using a blender (MBLR-4731, Mika-Dubai, UAE) and sieved using 300µm sieve. The powdered samples were then packaged in clean polythene bags then refrigerated at -20ºC prior to further analyses. Voucher specimens for the collected seaweeds are preserved at the Botany department in JKUAT.

3.4 Determination of the proximate composition of seaweeds

The moisture, crude fat, crude ash, crude fibre and crude protein of the algal samples were determined in triplicates using the standard methods by the Association of Official Analytical Chemists (AOAC, 2005). The moisture content was determined by oven drying method at 105°C where two grams of the seaweed sample was used. The ash content was analyzed by incineration method using an electric muffle furnace (Shimadzu KL-420, Japan). The crude fat content was extracted from five grams of the algal sample using the Soxhlet apparatus with petroleum ether as the solvent and determined gravimetrically after oven-drying at 70ºC for an hour.

The crude protein content of seaweeds was determined by the Kjeldahl method (AOAC, 2005) and a conversion factor of 6.25 was used to calculate the crude protein content from the nitrogen content. The crude fibre was determined by Hennenberg-Stohmann method (AOAC, 2005) where sequential digestion of seaweed samples with 1.25% H_2SO_4 and 1.25% NaOH using the fibreglass as a container. For drying and ashing, the crucible with the sample was dried in an oven for five hours at 105°C and ashed in the muffle furnace at 550°C for 16 hours. The weight of crucible with sample after drying and ashing was recorded and the crude fibre content was calculated. Nitrogen free extract (NFE) was determined as the weight difference using moisture, crude fat, crude ash, crude fibre and crude protein data (James, 1996).

3.5 Determination of mineral contents in seaweeds collected

Two grams of algal samples were ashed at 550ºC for 16 hours followed by dilution using 1N HCl. The quantification of calcium, magnesium, potassium, phosphorus, sodium, iron, zinc, copper, lead and cadmium was done by atomic absorption spectrometry (AAS) (Shimadzu AA-6200, Japan) according to AOAC methods (AOAC, 2005).

3.6 Formulation of wheat-seaweed biscuits

Wheat-seaweed biscuits were formulated by incorporating the seaweed with the highest iron and zinc content but lowest lead and cadmium contents. In this study, a modified formulation of wheat-seaweed biscuits by Manley (2011) was used. The ground seaweed was incorporated into biscuits at levels of 0-5% as shown in Table 3.1.

Table 3.1: Formulation of wheat-seaweed biscuits.

Ingredients	**Product (%)**					
	Control (0)	1	2	3	4	5
Ground seaweed (g)	0.0	5.0	10.0	15.0	20.0	25.0
Bakers flour (g)	500.0	495.0	490.0	485.0	480.0	475.0
Sugar (g)	250.0	250.0	250.0	250.0	250.0	250.0
Shortening (g)	150.0	150.0	150.0	150.0	150.0	150.0
Baking powder (g)	20.0	20.0	20.0	20.0	20.0	20.0
Salt (g)	2.5	2.5	2.5	2.5	2.5	2.5
Water (ml)	100.0	100.0	100.0	100.0	100.0	100.0

The biscuits were prepared according the standard AACC method 10-50D (AACC, 1995). All the ingredients mentioned in Table 3.1 were weighed accurately using an electric balance. The dry ingredients such as wheat flour, seaweed powder, salt and baking powder and were mixed up together. The shortening was softened in the mixer before adding sugar and the rest of the dry ingredients. The dough was kneaded and rolled up to thickness of 7mm.

Cookie cuts were pressed out of a 60mm diameter cutter. The dough cuts were arranged on baking sheets placed in stainless steel trays and baked in a preheated baking oven at 180ºC for 15 minutes. The biscuits were cooled to ambient temperature and packed and vacuum sealed in high density polyethylene bags.

3.7 Determination of spread factor of the baked biscuits

After cooling the seaweed-based biscuits for 30 minutes, they were laid edge to edge and the width measured. The biscuits were rotated 90º and re-measured to obtain an average width (*W*) of six biscuits in millimetres (mm). The biscuits were stacked on top of one another and measured for thickness. The biscuits were restacked in a different order and re-measured to get average thickness (*T*) of six cookies in mm. All measurements were read to the nearest 0.5 mm before dividing by six to obtain *W* (as-is) and *T* (as-is). The spread factor was calculated as ten multiplied by *W/T* ratio (AACC, 1995). Dough of good cookies flows much faster hence a greater spread factor is considered as suitable and most desirable cookie quality charcteristics (Cheng and Bhat, 2016).

3.8 Sensory evaluation of wheat-seaweed biscuits

Sensory evaluation of the wheat-seaweed biscuit was performed 12 hours after baking. Colour, taste, aroma, hardness and consumer preference were evaluated using a questionnaire (Appendix I) by 28 untrained panellists recruited from staff and graduate students of JKUAT. The samples were presented in clean plastic plates at room temperature, assigned a 4-digit random code. A 9-point hedonic scale (1 – dislike extremely to 9 – like extremely) with equivalent intervals between categories was used (Ihekoronye and Ngoddy, 1985).

3.9 Data analysis

Data was reported in means and standard error. The data was analysed using one-way analysis of variance (ANOVA) to determine the variations in chemical composition by species, month, algal divisions and site; determine the variations in physical properties and sensory attributes with increase in seaweed content in biscuits. Descriptive statistics, analysis of variance (ANOVA) and a general linear model (GLM) were done using SPSS for Windows release 20.0 software package. Duncan Multiple Range Test (DMRT) was used for separation of the means. Significance was determined at $p<0.05$.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Identification of seaweeds collected

Out of the 386 species of seaweeds (comprising 116, 56 and 214 species from the Families Chlorophyta, Phaeophyta and Rhodophyta, respectively) documented from the Kenya coast (Bolton *et al.*, 2007), 34 seaweeds were collected and identified in this study. The identified seaweeds comprised of thirteen (representing 38.24%), twelve (35.29%) and nine (26.47%) from the Classes Phaeophyta (Table 4.1), Chlorophyta (Table 4.2) and Rhodophyta (Table 4.3), respectively. The relatively high proportion of Chlorophyta (>30% of the flora) is similar to many tropical floras (Santelices *et al.*, 2009). In this study, the brown seaweeds collected were the majority which is contrary to studies by Price *et al.* (2006) and Santelices *et al.* (2009).

In this study, there was a wide diversity of seaweed species between sites ($p<0.01$) and months ($p<0.01$). This is in agreement with studies by Yarish and Wamukoya (1990) who reported a wide distribution and variety of seaweeds species in fifteen (15) sites along the Kenya coast during field surveys conducted between 1987 -1989. Variations in seaweed diversity with sites is probably due to changes in environmental conditions such as temperature, salinity and nutrients. Lapointe and Duke (1984) suggested that growth of seaweeds in the tropical coastal waters was generally limited by nutrient availability.

Table 4.1: Seaweed species from the Chlorophta Class collected from the Kenya coast.

Species	Site		
	Mkomani	Kibuyuni	Mtwapa*
Caulerpa racemosa	O	-	-
Caulerpa scapelliformis	J	-	-
Chaetomopha crassa	MJ	-	-
Codium dwarkense	O	-	-
Codium geopiorum	O	-	-
Enteromopha kylinii	J	J	JO
Enteromopha muscoides	MJ	JO	-
Halimeda macroloba	M	MJO	-
Ulva fasciata	MJO	-	-
Ulva lactuca	-	O	-
Ulva pulchra	-	J	-
Ulva reticulatum	MJO	JO	O

- Not found

* Sampling not done in March

M found in March

J found in July

O found in October

Table 4.2: Seaweed species from the Phaeophyta Class collected from the Kenya coast.

Species	**Site**		
	Mkomani	Kibuyuni	Mtwapa*
Cystoseira myrica	-	JO	JO
Cystoseira trinodis	-	M	-
Dictyota bartaynesiana	-	J	-
Dictyota cervicormis	-	O	-
Dictyota sp. (variety 1)	-	M	-
Dictyota sp. (variety 2)	-	M	-
Harmophysa cuneiformis	-	O	-
Hydroclathrus clathrus	-	JO	-
Padina tetrastromatica	JO	MJO	JO
Sargassum cristaefolium	JO	-	-
Sargassum oligocystum	MJ	MJO	JO
Sargassum sp.	O	M	-
Spatoglossum asperum	JO	-	-

- Not found

* Sampling not done in March

M found in March

J found in July

O found in October

Table 4.3: Seaweed species from the Rhodophyta Class collected from the Kenya coast

Species	**Site**		
	Mkomani	Kibuyuni	Mtwapa*
Acanthophora spicifera	O	MJO	JO
Chondrophycus papillosus	-	MJO	JO
Eucheuma denticulatum	MJO	-	-
Gracilaria arcuata	-	-	JO
Gracilaria salicornia	MJO	MJO	JO
Hypnea musciformis	MJO	-	-
Hypnea sp.	-	-	O
Laurencia intermedia	MJO	-	-
Soliera robusta	-	JO	-

- Not found

* Sampling not done in March

M found in March

J found in July

O found in October

Renaud and Luong-Van (2006) reported a wide diversity of species with seasons (summer 19 species, winter 20 species) although their study focused on macroalgae from Darwin Harbour, Australia. The differences in seaweed diversity was probably due to exposure to seasonal variations of abiotic factors that influenced metabolic functions such as photosysnthetic activity and growth rates; and levels of chemical constituents (Orduna-Rojas *et al.*, 2002). However, most of the Rhodophyta collected in this study were available throughout the sampling months (the SEM and NEM) in specific sampling sites. This is contrary to studies by Renaud and Luong-Van (2006) who reported that the red and brown seaweeds were predominant during summer and winter, respectively.

In this study, the green seaweeds collected were *Caulerpa* spp., *Chaetormorpha crassa*, *Codium* spp., *Enteromorpha* spp., *Halimeda macroloba* (Plate 4.1 A) and *Ulva* spp. *Enteromorpha kylinii* and *Ulva reticulatum* (Plate 4.1 B) were the only seaweed species found at the three sites in July and October 2013, respectively. The period April to October (SEM) is characterized by strong winds, low water and air temperatures, extensive cloud cover and high relative humidity (Mutai and Ward, 2000; Camberlin and Philippon, 2002). These conditions were favourable for the growth of *Enteromorpha kylinii* and *Ulva reticulatum* in July and October 2013, respectively.

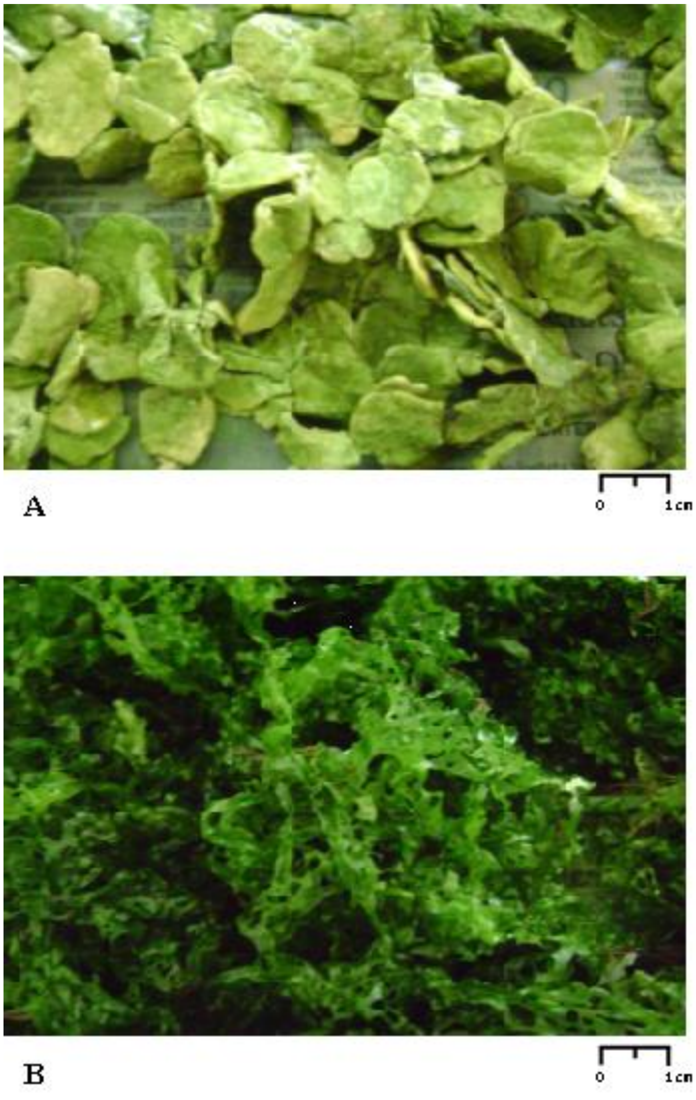


Plate 4.1: Green seaweeds (A) *Halimeda macroloba* and (B) *Ulva reticulatum*

Halimeda macroloba was found in Kibuyuni while *Ulva fasciata* and *U. reticulatum* in Mkomani in all the sampling months. This suggests that specific sites had favourable conditions for growth of specific seaweed species during the sampling months (Marinho-Soraino *et al.*, 2006). The green seaweeds identified in Mkomani were similar to those collected and identified by Yarish and Wamukoya (1990) in Mackenzie Point site (located at latitude 4° 3’ 49.3” S, longitude 39° 41’ 20.15” E). The seaweed habitat in Mackenzie Point is characterized by exposed and rocky topography (Yarish and Wamukoya, 1990) which could be similar to that in Mkomani.

Among the brown seaweeds, only *Cystoseira* spp., *Dictyota* spp., *Harmophysa cuneiformis*, *Hydroclathrus clathrus* (Plate 4.2 B), *Padina tetrastromatica*, *Sargassum* spp. and *Spatoglossum asperum* were collected. This is similar to brown seaweeds identified during the seaweed surveys of Kenya coastline conducted from 1987- 1989 (Yarish and Wamukoya, 1990). *Padina tetrastromatica* was found in the three sites in July and October 2013 while *Sargassum oligocystum* (Plate 4.2 A) was found in the three sites in July 2013.

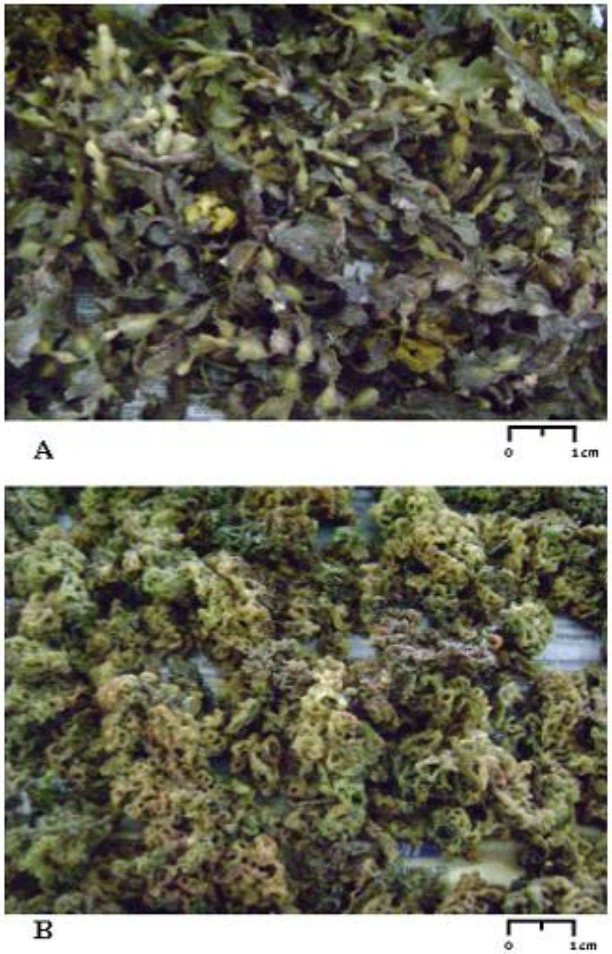


Plate 4.2: Brown seaweeds (A) *Sargassum oligocystum* and (B) *Hydroclathrus clathrus*

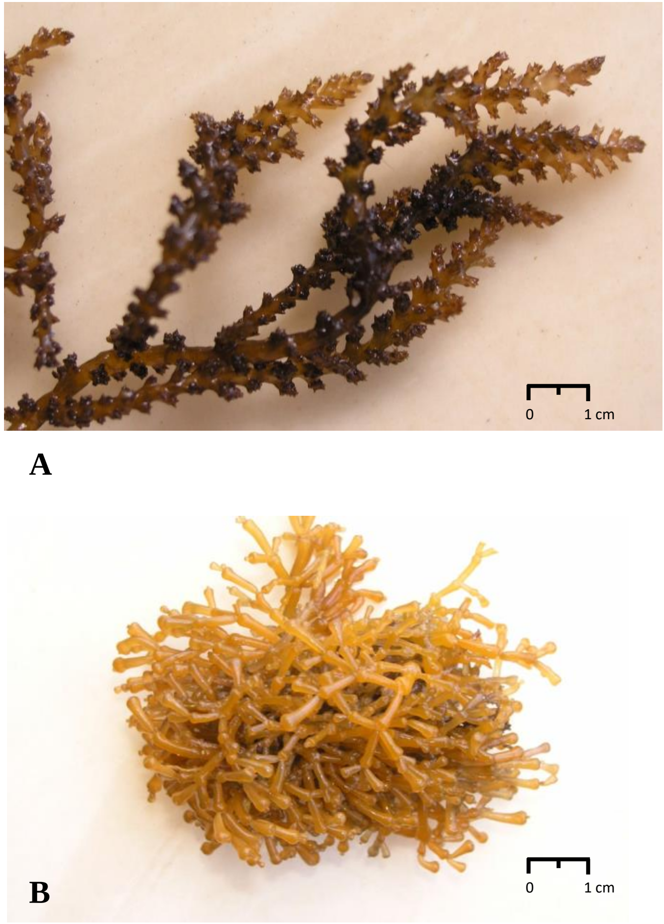


Plate 4.3: Red seaweeds (A) *Acanthophora spicifera* and (B) *Gracilaria salicornia*

In this study, the Kibuyuni had more diversity of brown seaweeds suggesting favourable ecological conditions for growth of brown seaweeds. Yarish and Wamukoya (1990) identified brown seaweeds were the dominated macroalgae in Shimoni site (latitude 4° 38’ 59.9” S, longitude 39° 22’ 59.9” E) which is near the Kibuyuni site. Shimoni site is characterized by rocky, coral reef, sand flats with seagrass meadows close to the sea shore (Yarish and Wamukoya, 1990) which could be similar to that in Kibuyuni.

During the sampling months of July and October 2013 (the SEM), there was significant number of seaweed species than in March 2013 (the NEM). This was also observed by Yarish and Wamukoya (1990) who noted that during the SEM period, there were significant standing crops of commercially important seaweeds. Kibuyuni site had wide diversity of seaweeds during the NEM unlike Mkomani and Mtwapa. Out of the 15 sites visited by Yarish and Wamukoya (1990), Shimoni was the only site with presence of seaweeds. This further suggests that Shimoni and Kibuyuni sites have similar topographical and ecological characteristics and are suitable seaweed habitats throughout the NEM and SEM period for potential seaweed farming.

The following red seaweeds were collected: *Acanthophora spicifera*, *Chondrophycus papillosus*, *Eucheuma denticulatum*, *Gracilaria* spp., *Hypnea* spp., *Laurencia* intermedia and *Soliera robusta*. Yarish and Wamukoya (1990) identified most of the seaweeds (identified in this study) except for *Soliera robusta* and *Laurencia intermedia*. *Acanthophora spicifera* (Plate 4.3 A) was found in the three sites in October 2013 which is one of the four species of *Acanthophora* reported by Bolton *et al.* (2007). Out of the four species of *Chondrophycus* (Bolton *et al.*, 2007), only *Chondrophycus papillosus* was found in the three sampling months in Kibuyuni. *Gracilaria salicornia* (Plate 4.3 B) was found in the three sampling months in Mkomani and Kibuyuni. This is one of the most common species of *Gracilaria* widely distributed along the Kenyan coastline (Yarish and Wamukoya, 1990; Bolton *et al.*, 2007). The seaweeds *Eucheuma denticulatum*, *Hypnea musciformis* and *Laurencia intermedia* were found in all the sampling months in Mkomani. Out of the two species of *Soliera* (Bolton *et al.*, 2007), only *Soliera robusta* was found in Kibuyuni in July and October 2013.

4.2 Proximate composition of seaweeds collected

The proximate composition of green, red and brown seaweeds is presented as % dry weight (dw) in Tables 4.4, 4.5 and 4.6, respectively. The highest proximate component from all the three classes was the nitrogen-free extract (NFE) (42.09 ± 0.83% dw) whereas the lowest component was crude fat (1.81 ± 0.04% dw; $p<0.05$). This is in agreement with studies by Fleurence (1999), Burtin (2003), and Muraguri *et al.* (2016) who determined that NFE (comprising of carbohydrates) and fats account for the highest and lowest chemical components, respectively. Dawes (1998) suggested that variation in chemical components in seaweeds is due to seasonal and ecological conditions. These conditions affect the synthesis of nutrients in seaweeds (Lobban and Harrison, 1994).

Table 4.4: Proximate composition (% dw) of green seaweeds collected along the Kenya coast.

Species	Crude fat	Crude fibre	Crude protein	Crude ash	Nitrogen-free extract
Caulerpa racemosa	1.91 ± 0.06^{efghi}	12.38 ± 0.10^{cdefgh}	5.17 ± 0.06^{m}	53.50 ± 0.12^{b}	27.04 ± 0.05^{hi}
Caulerpa scapelliformis	2.49 ± 0.03^{cdef}	12.61 ± 0.14^{cdefgh}	18.05 ± 0.08^{abc}	19.41 ± 0.26^{jkl}	47.54 ± 0.09^{abcdef}
Chaetomopha crassa	2.20 ± 0.08^{defg}	10.77 ± 0.08^{efgh}	10.92 ± 0.62^{fghi}	20.18 ± 0.11^{ijkl}	55.93 ± 0.52^{ab}
Codium dwarkense	1.54 ± 0.04^{ghij}	16.59 ± 0.54^{abcde}	7.32 ± 0.05^{ijklm}	69.94 ± 0.11^{a}	4.61 ± 0.50^{j}
Codium geopiorum	1.91 ± 0.06^{cdef}	16.34 ± 0.31^{bcdef}	14.86 ± 0.13^{cde}	37.96 ± 0.05^{cde}	28.92 ± 0.34^{ghi}
Enteromopha kylinii	1.42 ± 0.18^{hij}	14.20 ± 0.61^{cdefgh}	8.40 ± 0.95^{ijklm}	42.73 ± 2.40^{c}	33.24 ± 2.12^{efgh}
Enteromopha muscoides	1.83 ± 0.24^{fghi}	17.75 ± 2.80^{abc}	10.67 ± 0.72^{fghijk}	30.02 ± 1.48^{defghij}	39.73 ± 4.77^{bcdefgh}
Halimeda macroloba	1.95 ± 0.11^{efghi}	9.88 ± 1.45^{h}	5.28 ± 0.50^{m}	66.07 ± 0.60^{a}	16.82 ± 2.04^{ij}
Ulva fasciata	1.63 ± 0.18^{ghij}	10.74 ± 1.36^{fgh}	10.23 ± 0.62^{ghijk}	29.28 ± 5.07^{efghijk}	48.12 ± 6.48^{abcdef}
Ulva lactuca	1.65 ± 0.17^{ghij}	13.57 ± 0.14^{cdefgh}	14.99 ± 0.54^{cde}	23.67 ± 0.36^{hijkl}	46.11 ± 0.51^{abcdef}
Ulva pulchra	1.31 ± 0.04^{ij}	13.60 ± 0.40^{cdefgh}	10.67 ± 0.51^{fghijk}	22.04 ± 1.38^{hijkl}	52.38 ± 2.32^{abc}
Ulva reticulatum	1.34 ± 0.09^{ij}	11.86 ± 0.92^{defgh}	12.80 ± 0.84^{efgh}	18.60 ± 2.38^{jkl}	55.40 ± 2.42^{ab}

Values are given as means ± SE (n = 3). Values followed by different letters in superscript within columns are significantly different at $p<0.05$.

Table 4.5: Proximate composition (% dw) of brown seaweeds collected along the Kenya coast.

Species	**Crude fat**	**Crude fibre**	**Crude protein**	**Crude ash**	**Nitrogen-free extract**
Cystoseira myrica	1.58 ± 0.11^{ghij}	17.15 ± 1.33^{abcd}	8.16 ± 0.55^{ijklm}	41.38 ± 2.99^{cd}	31.75 ± 3.60^{fgh}
Cystoseira trinodis	2.08 ± 0.29^{efgh}	15.38 ± 0.95^{bcdefgh}	6.94 ± 1.14^{jklm}	33.64 ± 3.04^{cdefgh}	41.97 ± 4.57^{bcdefgh}
Dictyota bartaynesiana	3.10 ± 0.03^{bc}	12.95 ± 0.30^{cdefgh}	14.21 ± 0.06^{cde}	30.09 ± 0.02^{defghij}	39.65 ± 0.26^{bcdefgh}
Dictyota cervicormis	3.65 ± 0.09^{ab}	13.36 ± 0.96^{defg}	10.83 ± 0.06^{fghij}	36.54 ± 0.43^{cdef}	35.62 ± 0.36^{defgh}
Dictyota sp. 1	4.04 ± 0.00^{a}	14.11 ± 0.01^{cdefgh}	6.74 ± 0.00^{klm}	22.70 ± 0.11^{hijkl}	52.42 ± 0.11^{abc}
Dictyota sp. 2	4.21 ± 0.00^{a}	13.18 ± 0.02^{cdefgh}	1.71 ± 0.02^{n}	19.49 ± 0.00^{jkl}	61.42 ± 0.04^{a}
Harmophysa cuneiformis	1.82 ± 0.15^{fghi}	14.92 ± 1.51^{bcdefgh}	6.94 ± 0.61^{jklm}	33.19 ± 0.47^{cdefgh}	43.13 ± 1.52^{bcdefgh}
Hydroclathrus clathrus	1.62 ± 0.07^{ghij}	13.17 ± 0.29^{cdefg}	7.73 ± 0.54^{ijklm}	33.58 ± 8.01^{cdefgh}	43.90 ± 7.57^{bcdefg}
Padina tetrastromatica	1.91 ± 0.13^{efghi}	12.17 ± 0.39^{cdefgh}	7.62 ± 0.38^{ijklm}	41.24 ± 3.46^{cd}	37.07 ± 3.55^{cdefgh}
Sargassum cristaefolium	2.76 ± 0.11^{cd}	16.74 ± 0.12^{abcd}	9.41 ± 0.47^{hijkl}	24.64 ± 0.41^{ghijkl}	46.46 ± 0.10^{abcdef}
Sargassum oligocystum	2.56 ± 0.17^{cde}	15.12 ± 0.53^{bcdefgh}	7.56 ± 0.57^{ijklm}	$26.38 \pm 1.70^{efghijkl}$	48.37 ± 1.79^{abcde}
Sargassum sp.	2.19 ± 0.19^{defg}	13.34 ± 1.04^{cdefgh}	5.63 ± 0.55^{lm}	25.64 ± 0.39^{fghijkl}	53.20 ± 1.40^{abc}
Spatoglossum asperum	1.76 ± 0.12^{ghij}	16.35 ± 0.27^{bcdef}	17.17 ± 0.64^{bcd}	18.01 ± 1.56^{kl}	46.70 ± 1.03^{abcdef}

Values are given as means ± SE (n = 3). Values followed by different letters in superscript within columns are significantly different at $p<0.05$.

Table 4.6: Proximate composition (% dw) of red seaweeds collected along the Kenya coast.

Species	Crude fat	Crude fibre	Crude protein	Crude ash	Nitrogen-free extract
Acanthophora spicifera	1.39 ± 0.06^{hij}	13.15 ± 0.35^{cdefgh}	13.73 ± 0.96^{defg}	36.01 ± 2.05^{cdefg}	35.71 ± 2.59^{cdefgh}
Chondrophycus papillosus	1.52 ± 0.12^{ghij}	16.04 ± 0.68^{bcdefg}	9.61 ± 0.95^{hijk}	31.52 ± 1.23^{cdefghi}	41.30 ± 2.77^{bcdefgh}
Eucheuma denticulatum	1.82 ± 0.25^{fghi}	10.34 ± 1.98^{gh}	5.07 ± 0.46^{m}	36.21 ± 3.59^{cdefg}	46.56 ± 5.70^{abcdef}
Gracilaria arcuata	1.07 ± 0.16^{j}	19.89 ± 0.07^{ab}	13.79 ± 0.31^{defg}	16.51 ± 0.85^{l}	48.75 ± 0.45^{ij}
Gracilaria salicornia	1.47 ± 0.04^{hij}	12.52 ± 0.61^{cdefgh}	9.55 ± 0.71^{hijk}	29.10 ± 1.90^{efghijk}	47.37 ± 1.97^{bcdefgh}
Hypnea musciformis	1.38 ± 0.11^{hij}	14.30 ± 2.22^{cdefgh}	19.79 ± 1.44^{ab}	20.77 ± 1.36^{ijkl}	43.76 ± 5.00^{bcdefg}
Hypnea sp.	1.44 ± 0.05^{hij}	15.24 ± 0.13^{bcdefgh}	21.39 ± 0.06^{a}	$26.85 \pm 0.10^{efghijkl}$	35.07 ± 0.07^{efgh}
Laurencia intermedia	1.51 ± 0.15^{ghij}	21.73 ± 3.47^{a}	12.40 ± 1.16^{efgh}	30.32 ± 1.65^{defghij}	34.04 ± 6.13^{efgh}
Soliera robusta	1.57 ± 0.25^{ghij}	11.57 ± 0.04^{defgh}	10.84 ± 0.85^{fghij}	24.51 ± 1.56^{hijkl}	51.86 ± 2.67^{abcd}
Mean*	$^{e}1.81 \pm 0.04$	$^{c}14.08 \pm 0.26$	$^{d}10.09 \pm 0.26$	$^{b}31.94 \pm 0.78$	$^{a}42.09 \pm 0.83$

* Values of means of proximate component (for the 3 classes). Values given as means ± SE (n = 34). Values preceded by different letters in superscript within the row are significantly different at $p<0.05$.

Values are given as means ± SE (n = 3). Values followed by different letters in superscript within columns are significantly different at $p<0.05$.

Orduna-Rojas *et al.* (2002) reported that seasonal variations of abiotic factors such as water motion, light, temperature and salinity influenced key metabolic activities of seaweeds such as photosysnthetic activity and growth rates; and levels of chemical constituents. Banerjee *et al.* (2009) suggested significant positive correlation between salinity and surface water temperature and carbohydrate values. The study further reported an inverse relationship between carbohydrate and protein with temperature and salinity (Banerjee *et al.*, 2009). This corresponds to a pattern observed for several species of seaweeds (Mourandi-Givernaud *et al.*, 1993). The trend may be attributed to the positive role of light intensity, temperature and decrease of nitrogen for carbohydrate synthesis, while for the proteins these parameters act inversely (Rosemberg and Ramus, 1982; Rotem *et al.*, 1986).

The crude fat contents of *Dictyota* sp. 1 (4.04 ± 0.00% dw) and sp. 2 (4.21 ± 0.00% dw) were the highest whereas that of *Gracilaria arcuata* was the lowest (1.07 ± 0.16% dw; p<0.05). The lipid content of *Dictyota ciliolata* (7.8% dw) from Australia (Renaud and Luong-Van, 2006) were considerably higher than those found in species of *Dictyota* in this study. Studies by Muraguri *et al.* (2016) revealed that *Eucheuma denticulatum* (1.78% dw) and *Sargassum oligocystum* (0.46% dw) had the highest and lowest crude fat contents, respectively. These variations could be attributed to seaweed habitat, species and seasonality (Marinho-Soraino *et al.*, 2006).

The red seaweeds *Hypnea* sp. and *Hypnea musciformis* had the highest crude protein of 21.39 ± 0.06% dw and 19.79 ± 1.44% dw, respectively while the brown seaweed *Dictyota* sp. 2 had the lowest crude protein (1.71 ± 0.02% dw; p<0.05). Generally, Rhodophyta are characterized by higher protein content in comparison to Phaeophyta and Chlorophyta (Dawes, 1998). Similarly, Arori *et al.* (2019) recorded the highest protein content in *Hypnea cornuta* (22.4% dw) and *Hypnea musciformis* (21.55% dw). On the contrary, Muraguri *et al.* (2016) recorded the highest crude protein from the green seaweed *Ulva fasciata* (10.06% dw) while lowest from *Eucheuma denticulatum* (5.06% dw). These variations could be attributed to differences in seaweed species, seasonality and habitats (Marinho-Soraino *et al.*, 2006).

The crude ash contents of *Codium dwarkense* (69.94 ± 0.11% dw) and *Halimeda macroloba* (66.08 ± 1.05% dw) were the highest while that of *Gracilaria arcuata* was the lowest (16.51 ± 0.85% dw; $p<0.05$). Various authors have reported differences in crude ash. For instance, Muraguri *et al.* (2016) reported the highest and lowest ash content in *Eucheuma denticulatum* (27.13% dw) and *Hypnea musciformis* (12.79% dw), respectively. In this study, the crude ash content of *Hypnea musciformis* (20.77 ± 1.36% dw) were similarly to 21.57% dw reported by Siddique (2013) but higher than that reported by Muraguri *et al.* (2016). The species of *Halimeda* are highly calcareous in nature (Renaud and Luong-Van, 2006) which probably attributes to the high ash content. These variations in ash content could be attributed to temperature, pH and seaweed habitat which could have an influence on mineralization (Polat and Ozogul, 2009).

Dictyota sp. 2 had the highest NFE (61.42 ± 0.04% dw) ($p<0.05$) while the *Codium dwarkense* had the lowest NFE (4.61 ± 0.50% dw; $p<0.05$). From the Kenyan seaweeds studied by Muraguri *et al.* (2016), *Hypnea musciformis* had the highest carbohydrate content (73.07% dw) while *Laurencia intermedia* had lowest (57.93% dw). The figures were considerably higher compared to the NFE contents of *Hypnea musciformis* (43.76% dw) and *Laurencia intermedia* (34.04% dw) in this study. These variations could be attributed to differences in seasonality, habitats and species (Marinho-Soriano *et al.*, 2006).

The brown seaweeds had the highest crude fat of 2.32 ± 0.09% dw while Rhodophyta and Chlorophyta had the lowest crude fat of 1.50 ± 0.04% dw and 1.65 ± 0.06% dw, respectively ($p<0.05$) (Table 4.7). The highest percentages were in the species of *Dictyota* (See Table 4.5). This is in agreement with Renaud and Luong-Van (2006) who recorded the highest percentage of crude fat in Phaeophyta (4.0% dw), followed by Chlorophyta (3.1% dw) and then Rhodophyta (3.0% dw).

Table 4.7: Proximate composition (% dw) of seaweeds by algal divisions.

Algal divisions	Crude fat	Crude fibre	Crude protein	Crude ash	Nitrogen-free extract
Rhodophyta	1.50 ± 0.04^b	14.28 ± 0.53^a	11.56 ± 0.50^a	29.29 ± 0.89^b	43.37 ± 1.31^a
Chlorophyta	1.65 ± 0.06^b	13.30 ± 0.52^a	10.52 ± 0.42^a	35.08 ± 1.89^a	39.44 ± 1.80^a
Phaeophyta	2.26 ± 0.08^a	14.08 ± 0.26^a	8.20 ± 0.33^b	31.96 ± 1.22^b	43.05 ± 1.24^a

Values are given as means ± SE (n = 3). Values followed by different letters in superscript within columns are significantly different at $p<0.05$.

Similar to Renaud and Luong-Van (2006), there were no significant differences between crude fat contents of Chlorophyta and Rhodophyta. The crude protein contents of rhodophytes (11.56 ± 0.50%) and chlorophytes (10.52 ± 0.42%) were the highest whereas that of phaeophytes was the lowest (8.20 ± 0.33%; $p<0.05$). Similarly, Renaud and Luong-Van (2006) reported highest protein content from red seaweeds (8.0% dw), followed by Phaeophyta (6.9% dw) and lowest from Chlorophyta (4.8% dw) The chlorophytes had the highest crude ash (35.08 ± 1.89%) with the highest percentages in the heavily calcified *Halimeda macroloba* (See Table 4.4), followed by Rhodophyta (29.29 ± 0.89%) and Phaeophyta (31.96 ± 1.22%; $p<0.05$).

The *Dictyota* sp. 2 had the highest NFE but lowest crude protein. In this study, crude protein was negatively correlated to NFE. This suggests that seaweed species with high concentrations of NFE had low concentrations of crude protein. This trend may be related to nitrogen deficiency in macroalgae (Lobban and Harrison, 1994). Under long-term short supply of nitrogen, it is observed that an increase in total carbohydrate and a progressive decrease of the concentration of nitrogenous substances (protein, pigments, intracellular inorganic nitrogen, nucleic acids, etc.) over time occur, and this is a universal behaviour of seaweeds (Lobban and Harrison, 1994; Lourenço *et al.*, 2004).

In this study, there was no significant difference in carbohydrates (NFE) among the algal divisions. Previous studies showed that the maximum carbohydrate content was recorded in chlorophytes as opposed to phaeophytes and rhodophytes (Chakraborthy and Santra, 2008; Anantharaman *et al.*, 2013). Dhargalkar *et al.* (1980) from the Maharashtra coast in India noted the maximum value of carbohydrate content in rhodophytes than in phaeophytes and chlorophytes. The high content of carbohydrate in phaeophytes might be due to higher phycocolloid content in their cell walls (Dhargalkar *et al.*, 1980). Variation in NFE among seaweeds is related to species, habitat and seasonal changes (Dhargalkar *et al.*, 1980; Marinho-Soriano *et al.*, 2006).

The crude fat was the least chemical component among the seaweeds collected in this study. The crude fat of seaweed was less than 5% reported on crude fat of seaweeds in other works (Chan *et al.*, 1997) hence seaweeds are not considered to be good sources of crude fats. In this study, the crude fat contents of *Dictyota* sp. 1 and sp. 2 were the highest whereas that of *Gracilaria arcuata* was the lowest. However, a previous studies showed higher crude fat content in Australian *Dictyota ciliolata* (Renaud and Luong-Van, 2006) and Hawaiian *Dictyota acutiloba* and *Dictyota sandvicenis* (McDermid and Stuercke, 2003) than the *Dictyota* species in this study.

The crude fat content of *Gracilaria arcuata* was lower than that of *Gracilaria fisheri* (2.2% dw) and *Gracilaria tenuistipitata* (2.8% dw) from Thailand (Benjama and Masniyom, 2012) and Hawaiian *Gracilaria coronopifolia* (2.1% dw), *Gracilaria salicornia* (2.4% dw) and *Gracilaria parvispora* (2.8% dw) (McDermid and Stuercke, 2003). However, crude fat content of *Gracilaria arcuata* was higher than that of *Gracilaria cervicornis* (0.43% dw) and *Sargassum vulgare* (0.45% dw) of Brazil (Marinho-Soriano *et al.*, 2006) and *Sargassum oligocystum* of Kenya (Muraguri *et al.*, 2016). The crude fat contents of *Hypnea* sp. and *Hypnea musciformis* in this study were higher than those of *Hypnea charoides* and *Hypnea japonica* (<1.00% dw) (Wong and Cheung, 2000) and *Hypnea musciformis* (0.77% dw) (Muraguri *et al.*, 2016), but similar to *Hypnea pannosa* (1.56% dw) and *Hypnea musciformis* (1.27% dw) from Bangladesh (Siddique *et al.*, 2013).

Among the algal divisions, the phaeophytes had the highest crude fat while rhodophytes and chlorophytes had the lowest crude fat. This shows variations in crude fat contents among different species due to differences in classes among the seaweed species (Norziah and Ching, 2000) and climate and geography of development of the seaweed (Marinho-Soriano *et al.*, 2006).

The crude fibre fraction represents the indigestible portion of seaweeds. In this study, *Laurencia intermedia* had the highest crude fibre while *Halimeda macroloba* had the lowest crude fibre. *Hypnea musciformis* from this study had crude fibre content lower than that found in *Hypnea pannosa* (40.59% dw) and *Hypnea musciformis* (37.92% dw) from Bangladesh (Siddique *et al.*, 2013), but higher than that found in Hypnea musciformis (6.49% dw) (Muraguri *et al.*, 2016). *Laurencia intermedia* from this study had higher crude fibre content than that found in *Laurencia intermedia* (7.50% dw) (Muraguri *et al.*, 2016).

The crude fibre levels showed positive correlations with nitrogen and crude protein levels but showed a negative correlation with NFE implying the lower amount of crude fibre were probably due to the suitable environmental conditions such as temperature, salinity, water transparency for the synthesis of NFE and increased nutrient uptake (Wong and Cheung, 2000). Variations in the crude fibre of seaweeds can occur due to different species brought about by different growth stage and photosynthetic activity and season brought about by changing environmental parameters that influence photosynthesis and uptake of nutrients (Wong and Cheung, 2000; Siddique *et al.*, 2013).

The crude protein contents of seaweed species in this study were highest in *Hypnea* sp. and *Hypnea musciformis* and were within the range for red seaweeds of 10–47% dw (Fleurence, 1999). The crude protein content of *Hypnea* sp. and *Hypnea musciformis* are comparable to 20% dw of *Hypnea* species in Brazil but lower than 47% dw from Korean *Ulva*. Variations in the crude protein content of seaweeeds can occur due to differences among species and season (Fleurence, 1999). The crude protein of *Hypnea* sp. and *Hypnea musciformis* were higher than that of *Sargassum polycystum* (5.4% dw) (Matanjun *et al.*, 2009), Brazilian *Gracilaria domingensis* (6.2% dw) and *Gracilaria birdiae* (7.1% dw) (Gressler *et al.*, 2010), and it was closely related to *Hypnea pannosa* (16.31% dw) from Bangladesh (Siddique *et al.*, 2013). In this study, *Gracilaria* species had mean crude protein content within the crude protein range (7–13% dw) for most *Gracilaria* species (Briggs and Smith, 1993).

Ulva lactuca had crude protein content higher than that of Iranian *Ulva lactuca* (10.69% dw) (Tabarsa *et al.*, 2012), Tunisian *Ulva rigida* (7.31% dw) (Frikha *et al.*, 2011) and *Ulva fasciata* (10.06% dw) (Muraguri *et al.*, 2016), but similar to that of *Ulva pertusa* (15.4% dw) and *Ulva intestinalis* (17.9% dw) from Bangladesh (Benjama and Masniyom, 2011) but lower than that of *Ulva lactuca* (4.2% dw) found in the Philipines (Portugal *et al.*, 1983). In this study, all the seaweed species were lower in crude protein than those of other seaweed species such as *Porphyra tenera* (47% dw) and *Palmaria palmata* (35% DW) (Fleurence, 1999). Protein content varied among different genera and also in different species of the same genus (Dhargalkar *et al.*, 1980).

The crude ash content obtained in this study fit well within the wide range of 8–40% dw in seaweeds (Mabeau and Fleurence, 1993). Generally, seaweeds have high crude ash because of their cell wall polysaccharides and proteins contain anionic carboxyl, sulfate, and phosphate groups that are excellent binding sites for metal retention (Davis *et al.*, 2003) which invariably indicates the presence of appreciable amounts of diverse mineral components (Matanjun *et al.*, 2009). In this study, *Codium dwarkense* had the highest crude ash as compared to its closely related *Codium geppiorum* in the same study implying differences among species in the same genera.

Gracilaria arcuata had the least crude ash which was lower than that of *Gracilaria fisheri* (21.2% dw) and *Gracilaria tenuistipitata* (17.0% dw) from Thailand (Benjama and Masniyom, 2011) and Brazilian *Gracilaria domingensis* (23.8% dw) and *Gracilaria birdiae* (22.5% dw) (Gressler *et al.*, 2010). *Ulva* *fasciata* had a crude ash content similar to that of *Ulva pertusa* (27.2% dw) and *Ulva intestinalis* (27.6% dw) from Thailand (Benjama and Masniyom, 2012). Differences in ash content within species could be due to different habitats where they grow which may have varying concentration of inorganic compounds and salts in water environment and differing methods of mineralization in the species influenced by temperatures and pH (Mendis and Kim, 2011; Polat and Ozogul, 2009).

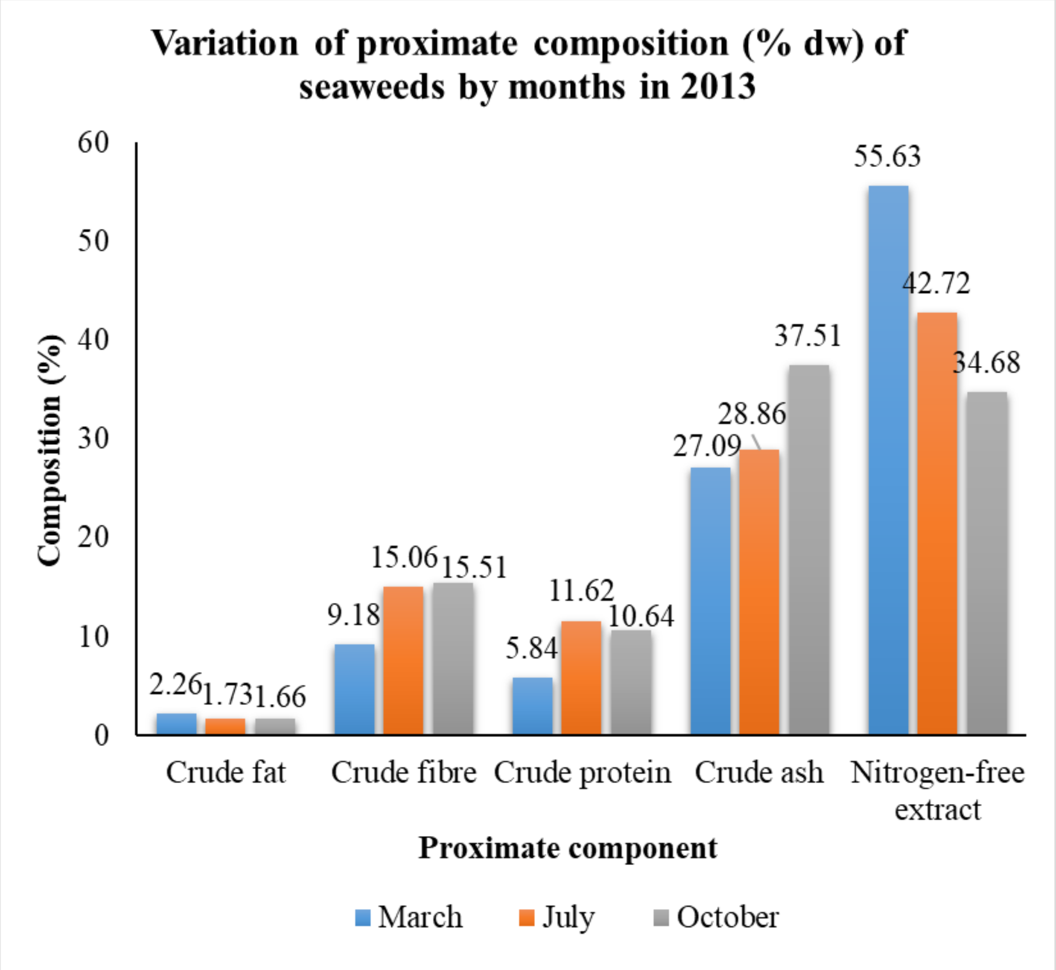


Figure 4.1: Variation of proximate composition (% dw) of seaweeds by months in 2013.

The chemical composition of seaweed species varied with the month as shown in Figure 4.1. The seaweeds collected in March had the highest crude fat of 2.26 ±

0.13% while those collected in the months of July and October had the lowest crude fat of 1.73 ± 0.05 and 1.66 ± 0.05%, respectively ($p<0.05$). The crude fibre contents of the seaweeds collected in October and July were the highest, 15.51 ± 0.35 and 15.06 ± 0.34%, respectively, while those in March had the lowest (9.18 ± 0.53%; $p<0.05$). The highest crude protein was obtained from seaweeds collected in July (11.62 ± 0.38%) and October (10.64 ± 0.36%) while those in March had the lowest (5.84 ± 0.43%; $p<0.05$). The seaweeds collected in October had the highest crude ash of 37.51 ± 1.24% while those collected in July and March had the lowest crude ash of 28.86 ± 0.96 and 27.09 ± 1.95%, respectively ($p<0.05$). The seaweeds collected in March had the highest NFE of 55.63 ± 1.83% while those collected in October had the lowest NFE of 34.68 ± 1.17% ($p<0.05$).

The seaweeds collected in March had higher highest nitrogen-free extract (NFE) values while the lowest values were obtained from those collected in July and October probably due to differences in abiotic factors that affect photosynthetic activity of seaweeds. The month of March is within northeast Monsoon (NEM) and is characterized by high seawater surface temperatures, averaging 28.4°C (maximum 29°C) due to long duration of sunlight while July and October fall under the southeast Monsoon (SEM) (Mutai and Ward, 2000; Camberlin and Philippon, 2002). Synthesis of carbohydrates (NFE) has been reported to be favoured by the intensity of light, temperature and decrease of nitrogen (Bird *et al.*, 1990; Dawes *et al.*, 1974) while for crude protein these parameters acted inversely (Rosemberg and Ramus, 1982). It appears that the high NFE in the month of March was probably due to high light intensity, increased temperatures and decrease in nitrogen. In this study, NFE and crude fat were both highest in seaweeds collected in March implying the environmental conditions that favour photosynthesis favoured crude fat synthesis (Bird *et al.*, 1990). Notably, crude fat was positively correlated to NFE. This is in contrast with findings reported by Sanchez-Machado *et al.* (2004) and Khairy and El-Shafay (2013) that as the temperature increased, the crude fat content decreased and remained almost stable until the end of the growing season while carbohydrates increased.

In this study, the seaweeds collected in the cooler months of July and October, had high crude protein and fibre whereas those in March had the lowest crude protein and fibre. Previous studies have suggested that plants exhibiting faster growth rates showed a higher ratio of crude protein to carbohydrate and vice-versa (Dawes *et al.*, 1974; Bird *et al.*, 1990; Marinho-Soriano *et al.*, 2006). Synthesis of carbohydrates seemed to be favoured by both, the intensity of light and temperature while decreasing the proteins. The seaweeds collected in October had the highest crude fibre and protein probably due to favourable environmental conditions such as high salinity and low water surface temperatures which in turn suppressed photosynthesis thus low NFE. The seaweeds collected in October had the least crude fat content due to unfavourable environmental conditions (Mutai and Ward, 2000; Camberlin and Philippon, 2002). Rosemberg and Ramus (1982) related the carbohydrate synthesis to periods of maximum growth, increased photosynthetic activity and a reduction in nitrogen and protein contents.

The chemical composition of the seaweeds collected varied among the three sites (Figure 4.2). The seaweeds collected in Kibuyuni had the highest crude fat content of 2.01 ± 0.08% while those in Mtwapa had the lowest crude fat of 1.58 ± 0.05% (p<0.05). Seaweeds collected in Mtwapa had the highest crude fibre of 15.45 ± 0.34% while those in Mkomani and Kibuyuni had the least crude fibre of 13.58 ± 0.50 and 13.77 ± 0.40%, respectively (p<0.05). The seaweeds collected in Mtwapa had the highest crude protein of 12.16 ± 0.43% while those in Kibuyuni had the lowest and crude protein of 8.25 ± 0.33% (p<0.05). There was no significant difference in crude ash among the sites (p>0.05). Seaweeds collected in Kibuyuni had the highest NFE of 44.04 ± 1.47% while those in Mtwapa had the lowest NFE of 39.08 ± 1.09% (p<0.05).

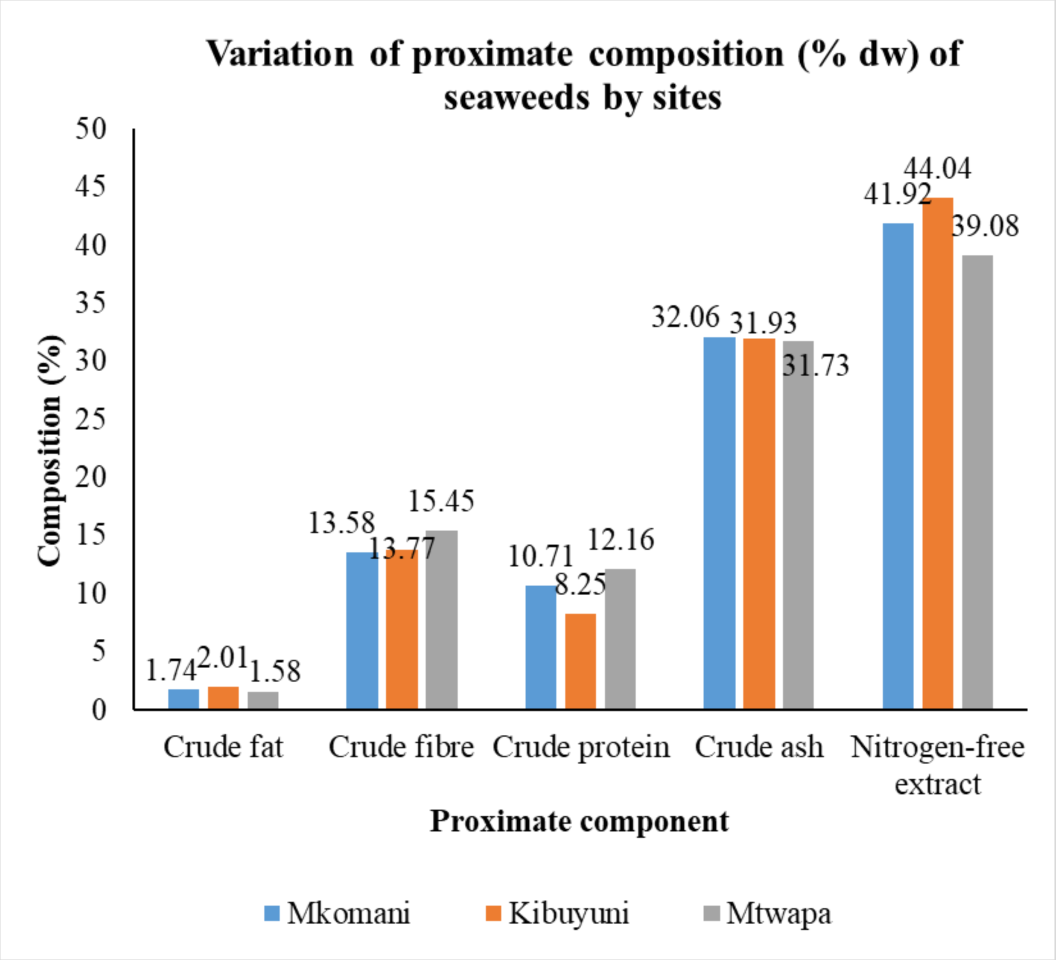


Figure 4.2: Variation of proximate composition (% dw) of seaweeds by sites

The Kenya coast is characterized by uncovered or almost uncovered reef platform. The surface of the reef platform is very uneven with parts completely uncovered in Kibuyuni, parts with shallow water in Mkomani and parts with larger and smaller pools in Mtwapa during the low tide. The uneven character of the reef surface together with the existence of many pools could possibly explain the differences in chemical composition. The tissue nitrogen content is a measure of the nutrients (nitrogen) in seawater. Seaweeds exposed to sunlight had lower nitrogen contents as opposed to those partially or completely covered by a film of seawater implying that nitrogen content varied with exposure. The crude protein content is an expression of nitrogen content thus its composition varies as well. This study supports the findings of Dawes *et al.* (1974) and Hurtado-Ponce (1995) that intense sunlight causes degradation of protein and subsequent bleaching. The crude protein levels were positively correlated to nitrogen content implying that there was higher nitrogen content in waters from Mtwapa and Mkomani sites than Kibuyuni. The fluctuation in the protein values in all the three sites could probably be explained by variation in environmental conditions such as nutrients (Dawes, 1998; Burtin, 2003).

4.3 Mineral composition of seaweeds collected

The mineral contents of the seaweeds collected are shown in Tables 4.8 to 4.13 in dry weight basis (dw). The major mineral was magnesium with a mean value of 1523.45 ± 66.93 mg/100g while the lowest was phosphorus with a mean value of 2.51 ± 0.08 mg/100g ($p<0.05$) (Table 4.10). The highest magnesium content was obtained from *Ulva pulchra* (3152.01 ± 4.61 mg/100g; $p<0.05$) (Table 4.8) while the lowest magnesium content was found in *Eucheuma denticulatum* (556.36 ± 65.48 mg/100g; $p<0.05$) (Table 4.10).

Table 4.8: Mineral (Na, K, Ca, Mg and P) composition (mg/100g dw) of green seaweeds along the Kenya coast.

Species	Na	K	Ca	Mg	P
Caulerpa scapelliformis	37.93 ± 6.69^{fg}	28.36 ± 1.54^{hi}	3005.68 ± 26.74^{a}	817.51 ± 11.69^{efg}	1.51 ± 0.02^{fghij}
Chaetomopha crassa	133.93 ± 0.80^{bcde}	223.95 ± 2.62^{bcde}	1006.31 ± 18.35^{cdefg}	828.41 ± 8.23^{efg}	2.59 ± 0.01^{bcdefg}
Enteromopha kylinii	63.14 ± 7.22^{defg}	25.93 ± 3.08^{hi}	1737.16 ± 293.63^{bc}	2921.78 ± 42.74^{ab}	2.67 ± 0.14^{bcdefg}
Enteromopha muscoides	50.00 ± 10.84^{efg}	39.33 ± 2.46^{ghi}	1007.43 ± 188.82^{cdefg}	2303.92 ± 272.94^{abc}	2.72 ± 0.06^{bcdefg}
Halimeda macroloba	27.21 ± 6.22^{fg}	38.99 ± 8.21^{ghi}	2329.72 ± 516.04^{ab}	804.62 ± 139.14^{efg}	3.43 ± 0.62^{b}
Ulva fasciata	70.38 ± 24.90^{cdefg}	29.00 ± 3.20^{hi}	912.95 ± 146.37^{defgh}	2208.41 ± 451.93^{bcd}	2.01 ± 0.81^{cdefghi}
Ulva lactuca	44.76 ± 5.00^{efg}	25.59 ± 0.57^{hi}	516.44 ± 6.16^{defgh}	3013.98 ± 43.54^{ab}	1.17 ± 0.00^{ij}
Ulva pulchra	51.21 ± 0.28^{efg}	30.71 ± 0.36^{hi}	1004.77 ± 5.28^{cdefg}	3152.01 ± 4.61^{a}	0.45 ± 0.01^{j}
Ulva reticulatum	47.35 ± 9.61^{efg}	22.33 ± 1.23^{i}	614.93 ± 42.73^{defgh}	2207.95 ± 692.76^{bcd}	1.54 ± 0.21^{fghij}

Values are given as means ± SE (n = 3). Values followed by different letters in superscript within columns are significantly different at $p<0.05$.

Table 4.9: Mineral (Na. K, Ca, Mg and P) composition (mg/100g dw) of brown seaweeds along the Kenya coast.

Species	**Na**	**K**	**Ca**	**Mg**	**P**
Cystoseira myrica	101.97 ± 11.55^{bcdef}	123.37 ± 909^{efghi}	849.38 ± 64.01^{defgh}	1719.99 ± 26.69^{cde}	2.62 ± 0.08^{bcdefgh}
Cystoseira trinodis	25.16 ± 0.04^{fg}	157.60 ± 3.12^{defgh}	358.21 ± 2.18^{fgh}	1416.02 ± 10.71^{cdefg}	3.10 ± 0.02^{bcde}
Dictyota bartaynesiana	100.86 ± 0.42^{bcdefg}	132.89 ± 2.15^{defghi}	374.79 ± 1.25^{fgh}	1337.96 ± 7.44^{cdefg}	2.70 ± 0.01^{bcdefg}
Dictyota sp. 1	48.26 ± 1.17^{efg}	1342.35 ± 72.82^{a}	238.96 ± 0.53^{gh}	1410.42 ± 12.11^{cdefg}	5.23 ± 0.02^{a}
Dictyota sp. 2	89.96 ± 0.96^{cdefg}	113.66 ± 2.01^{efghi}	1199.27 ± 1.42^{cd}	1580.70 ± 16.48^{cdef}	5.66 ± 0.02^{a}
Harmophysa cuneiformis	153.03 ± 1.35^{bcd}	253.60 ± 0.94^{bcd}	869.09 ± 5.48^{defgh}	1271.96 ± 3.04^{defg}	1.69 ± 0.01^{fghij}
Hydroclathrus clathrus	76.11 ± 0.30^{cdefg}	79.71 ± 1.18^{fghi}	1184.17 ± 11.71^{cde}	1402.82 ± 6.06^{cdefg}	2.79 ± 0.01^{bcdef}
Padina tetrastromatica	28.30 ± 1.31^{fg}	36.06 ± 10.31^{hi}	1069.63 ± 151.94^{cdef}	2151.48 ± 108.33^{bcd}	2.83 ± 0.10^{bcdef}
Sargassum cristaefolium	159.29 ± 3.93^{bc}	165.83 ± 3.10^{defg}	234.05 ± 2.19^{gh}	1139.65 ± 15.51^{efg}	2.14 ± 0.04^{bcdefghi}
Sargassum oligocystum	104.24 ± 22.27^{bcdef}	189.26 ± 26.47^{cdef}	430.72 ± 45.91^{defgh}	1256.37 ± 110.74^{defg}	2.50 ± 0.25^{bcdefghi}
Sargassum sp.	6.81 ± 0.07^{g}	27.51 ± 0.44^{hi}	719.81 ± 9.71^{defgh}	1058.46 ± 11.59^{efg}	2.09 ± 0.02^{bcdefghi}
Spatoglossum asperum	81.49 ± 3.11^{cdefg}	34.80 ± 0.39^{hi}	838.78 ± 8.22^{defgh}	2783.64 ± 15.34^{ab}	1.33 ± 0.01^{ghij}

Values are given as means ± SE (n = 3). Values followed by different letters in superscript within columns are significantly different at $p<0.05$.

Table 4.10: Mineral (Na. K, Ca, Mg and P) composition (mg/100g dw) of red seaweeds along the Kenya coast.

Species	Na	K	Ca	Mg	P
Acanthophora spicifera	111.04 ± 23.35^{bcdef}	165.92 ± 13.01^{defg}	379.72 ± 61.85^{fgh}	1222.54 ± 57.15^{defg}	2.28 ± 0.06^{bcdefghi}
Chondrophycus papillosus	118.33 ± 22.17^{bcdef}	154.59 ± 9.91^{defghi}	507.41 ± 52.81^{defgh}	1481.98 ± 56.79^{cdefg}	2.57 ± 0.11^{bcdefgh}
Eucheuma denticulatum	112.54 ± 44.53^{bcdef}	222.79 ± 64.02^{bcde}	552.47 ± 19.80^{defgh}	556.36 ± 65.48^{g}	1.83 ± 0.53^{defghi}
Gracilaria arcuate	68.92 ± 0.70^{cdefg}	76.81 ± 1.24^{fghi}	277.62 ± 1.20^{fgh}	748.79 ± 3.38^{efg}	1.22 ± 0.01^{hij}
Gracilaria Salicornia	85.14 ± 18.30^{cdefg}	301.00 ± 52.32^{bc}	320.39 ± 30.28^{fgh}	638.70 ± 67.90^{fg}	2.44 ± 0.26^{bcdefghi}
Hypnea musciformis	83.73 ± 35.59^{cdefg}	34.79 ± 10.54^{hi}	605.63 ± 162.44^{defgh}	833.94 ± 57.13^{efg}	3.35 ± 0.37^{bc}
Hypnea sp.	360.92 ± 5.14^{a}	73.17 ± 1.04^{fghi}	2836.45 ± 12.99^{a}	974.44 ± 1.69^{efg}	1.78 ± 0.00^{efghij}
Laurencia intermedia	58.22 ± 22.74^{efg}	35.12 ± 6.63^{hi}	396.75 ± 50.22^{efgh}	1144.83 ± 196.26^{efg}	3.20 ± 0.54^{bcd}
Soliera robusta	189.65 ± 2.17^{b}	326.92 ± 2.14^{b}	193.28 ± 2.44^{h}	1669.37 ± 13.64^{cde}	2.48 ± 0.01^{bcdefghi}
Mean*	83.83 ± 5.46	137.16 ± 14.63	837.84 ± 57.90	1523.45 ± 66.93	2.51 ± 0.08

*Values of means of the mineral element (Tables 4.8 to 4.10).

Values are given as means ± SE (n = 3). Values followed by different letters in superscript within columns are significantly different at $p<0.05$.

The magnesium content for *Ulva pulchra* was significantly higher than 140 mg/100g in *Ulva reticulatum* from Thailand (Ratana-arporn and Chirapart, 2006), 429 mg/100g in *Ulva fasciata* from Kenya (Muraguri *et al.*, 2016) and slightly higher than 3098.1 mg/100g in *Ulva intestinalis* but lower than 3670 mg/100g in *Ulva pertusa*, both from Southern Thailand (Benjama and Masniyom, 2011). The magnesium content for *Eucheuma denticulatum* reported here was higher than 271 mg/100g obtained in *Eucheuma cottonii* from Malaysia (Matanjun *et al.*, 2009) and *Eucheuma denticulatum* from Kenya (Muraguri *et al.*, 2016). In this study, the chlorophytes, *Ulva pulchra* (3152.01 ± 4.61 mg/100g) and *Caulerpa scapelliformis* (3005.68 ± 26.74 mg/100g) had the highest magnesium and calcium contents, respectively. This is in contrast with Ruperez (2002) who found magnesium and calcium to be the most abundant elements in phaeophytes and rhodophytes studied in Spain.

In this study, the highest calcium content was found in *Caulerpa scapelliformis* (3005.68 ± 26.74 mg/100g; $p<0.05$) while the lowest calcium content was obtained from *Soliera robusta* (193.28 ± 2.44 mg/100g; $p<0.05$). The calcium content obtained in *Caulerpa scapelliformis* was considerably higher than 1874.74 mg/100g reported on *Caulerpa lentilifera* from Malaysia (Matanjun *et al.*, 2009) and 630 mg/100g from Thailand (Ratana-arporn and Chirapart, 2006). The calcium contents reported here for *Eucheuma denticulatum* were higher than 422 mg/100g obtained in *Eucheuma* spp. from South China (Krishnaiah *et al.*, 2008) and 329.69 mg/100g in *Eucheuma cottonii* from Malaysia (Matanjun *et al.* 2009) but lower than those reported in *Eucheuma denticulatum* (1536.93 mg/100g) from Kenya (Muraguri *et al.*, 2016). Minerals such as calcium accumulate more in seaweeds compared to most terrestrial plants (MacArtain *et al.*, 2007). The calcium content obtained from the studied seaweed species was considerably higher compared to common vegetables such as lettuce (35 mg/100g), cabbage (40 mg/100g) and spinach (99 mg/100g) (USDA, 2016). The seaweeds from this study showed potential for utilization as vegetable supplements in the human diet. There were significant differences in mineral composition among the seaweed species. However, differences in seasons and habitats could bring about variation in mineral content in seaweeds (Ruperez, 2002; Mendis and Kim, 2011; Polat and Ozogul, 2009).

Dictyota sp. 1 had the highest potassium content (1342.35 ± 72.82 mg/100g; $p<0.05$) while *Ulva reticulatum* had the lowest potassium content (22.33 ± 1.23 mg/100g; $p<0.05$). The potassium content of *Ulva reticulatum* reported here was considerably lower than 2412.02 mg/100g obtained in *Ulva lactuca* from South Iran (Tabarsa *et al.*, 2011), and 1224.1 and 2538.6 mg/100g from *Ulva pertusa* and *Ulva intestinalis*, respectively, from Southern Thailand (Benjama and Masniyom, 2011). The potassium content of *Dictyota* sp. 1, a phaeophyte, was lower than 8371.23 mg/100g obtained in *Sargassum polycystum*, also a phaeophyte, from Malaysia (Matanjun *et al.*, 2009). This shows differences in mineral composition of seaweeds within the same algal groups (Ruperez, 2002; Polat and Ozogul, 2009; Mendis and Kim, 2011).

Hypnea sp. had the highest sodium content (360.92 ± 5.14 mg/100g; $p<0.05$) whereas *Sargassum* sp. had the lowest sodium content (6.81 ± 0.07 mg/100g; $p<0.05$). In this study, the sodium content of *Hypnea* sp. was significantly higher than 83.73 ± 35.59 mg/100g obtained from *Hypnea musciformis* (current study), 110.3 and 150.0 mg/100g from *Hypnea musciformis* and *Hypnea* sp., respectively, from the Bangladesh Coast (Khan *et al.*, 2016) suggesting differences within same genera and species. The sodium content of *Sargassum* spp. reported in this study were considerably lower than 1362.13mg/100g in *Sargassum polycystum* studied by Matanjun *et al.* (2009). The sodium content of *Sargassum cristaefolium* reported here was higher than 144.4 mg/100g in *Sargassum oligocystum* reported by Khan *et al.* (2016).

The phosphorus contents of *Dictyota* sp. 1 (5.23 ± 0.02 mg/100g) and sp. 2 (5.66 ± 0.02 mg/100g) were the highest whereas that of *Ulva pulchra* was the lowest (0.45 ± 0.01 mg/100g; $p<0.05$). In this study, the phosphorus contents of the *Dictyota* sp. 1 and sp. 2 were higher than that of *Dictyota bartaynesiana* ($p<0.05$)

indicating that there were differences in mineral content among seaweeds of the same genus. The phosphorus content of the *Ulva pulchra* reported here were significantly lower than 180 mg/100g obtained in *Ulva reticulatum* from Thailand (Ratana-arporn and Chirapart, 2006), 177.0 and 271.9 mg/100g from *Ulva pertusa* and *Ulva intestinalis*, respectively, from Southern Thailand (Benjama and Masniyom, 2011). The variation in mineral composition could be attributed to the difference in species and geographical location (Rao *et al.*, 2007).

Table 4.11: Mineral (Fe, Zn, Cu, Pb and Cd) composition (mg/100g dw) of green seaweeds along the Kenya coast.

Species	**Fe**	**Zn**	**Cu**	**Pb**	**Cd**
Caulerpa scapelliformis	150.19 ± 0.40^{ghij}	9.39 ± 0.20^{c}	6.43 ± 0.43^{b}	0.24 ± 0.00^{cdefg}	2.51 ± 0.27^{cdefg}
Chaetomopha crassa	151.39 ± 1.36^{ghij}	7.11 ± 0.25^{c}	5.65 ± 0.37^{b}	0.23 ± 0.02^{cdefg}	1.50 ± 0.30^{defghi}
Enteromopha kylinii	280.66 ± 26.30^{efgh}	21.08 ± 2.58^{bc}	11.47 ± 1.88^{b}	0.34 ± 0.02^{bcd}	2.35 ± 0.24^{cdefgh}
Enteromopha muscoides	348.08 ± 40.30^{def}	31.18 ± 8.48^{bc}	7.65 ± 0.45^{b}	0.18 ± 0.02^{cdefg}	2.22 ± 0.49^{cdefghi}
Halimeda macroloba	82.59 ± 5.77^{ij}	81.95 ± 21.02^{a}	15.85 ± 2.88^{b}	0.32 ± 0.09^{bcdef}	3.70 ± 0.95^{c}
Ulva fasciata	68.63 ± 10.25^{ij}	49.25 ± 15.55^{abc}	9.92 ± 2.63^{b}	0.21 ± 0.01^{cdefg}	0.22 ± 0.11^{i}
Ulva lactuca	146.47 ± 4.16^{ghij}	19.94 ± 0.27^{bc}	11.78 ± 0.22^{b}	0.12 ± 0.02^{defg}	1.22 ± 0.33^{efghi}
Ulva pulchra	146.62 ± 0.57^{ghij}	13.21 ± 0.52^{c}	14.83 ± 0.46^{b}	0.08 ± 0.00^{fg}	2.74 ± 0.38^{cdef}
Ulva reticulatum	124.54 ± 25.94^{hij}	59.71 ± 11.80^{ab}	106.41 ± 47.89^{a}	0.21 ± 0.06^{cdefg}	1.22 ± 0.30^{efghi}

Values are given as means ± SE (n = 3). Values followed by different letters in superscript within columns are significantly different at $p<0.05$.

Table 4.12: Mineral (Fe, Zn, Cu, Pb and Cd) composition (mg/100g dw) of brown seaweeds along the Kenya coast.

Species	Fe	Zn	Cu	Pb	Cd
Cystoseira myrica	553.93 ± 94.85^{c}	28.72 ± 1.68^{bc}	18.68 ± 1.95^{b}	0.50 ± 0.02^{b}	3.70 ± 0.21^{c}
Cystoseira trinodis	45.81 ± 0.74^{j}	30.41 ± 1.53^{bc}	9.96 ± 0.84^{b}	0.05 ± 0.00^{g}	0.56 ± 0.04^{ghi}
Dictyota bartaynesiana	466.07 ± 1.21^{cd}	17.73 ± 0.30^{bc}	10.34 ± 0.27^{b}	0.05 ± 0.01^{g}	2.48 ± 0.23^{cdefg}
Dictyota sp. 1	292.72 ± 0.88^{hij}	23.67 ± 1.58^{bc}	4.34 ± 0.57^{b}	0.12 ± 0.00^{defg}	0.51 ± 0.04^{ghi}
Dictyota sp. 2	314.25 ± 3.87^{defg}	50.69 ± 1.40^{abc}	39.14 ± 1.15^{b}	0.02 ± 0.00^{g}	0.20 ± 0.04i
Harmophysa cuneiformis	143.46 ± 5.07^{ghij}	17.10 ± 0.30^{bc}	17.12 ± 0.28^{b}	0.34 ± 0.01^{bcde}	3.46 ± 0.41^{cd}
Hydroclathrus clathrus	973.07 ± 2.65^{b}	21.44 ± 0.48^{bc}	11.94 ± 0.48^{b}	0.72 ± 0.00^{a}	10.21 ± 0.41^{a}
Padina tetrastromatica	365.83 ± 51.73^{def}	40.01 ± 4.32^{bc}	26.54 ± 6.25^{b}	0.40 ± 0.08^{bc}	3.72 ± 0.58^{c}
Sargassum cristaefolium	159.42 ± 1.44^{ghij}	26.28 ± 0.43^{bc}	4.05 ± 0.30^{b}	0.09 ± 0.01^{fg}	0.69 ± 0.40^{fghi}
Sargassum oligocystum	234.26 ± 32.01^{fghij}	29.97 ± 3.46^{bc}	10.25 ± 1.14^{b}	0.22 ± 0.07^{cdefg}	0.99 ± 0.18^{efghi}
Sargassum sp.	2226.91 ± 31.29^{a}	15.03 ± 1.06^{c}	11.43 ± 0.59^{b}	0.06 ± 0.01^{g}	0.90 ± 0.05^{efghi}
Spatoglossum asperum	98.77 ± 1.14^{hij}	25.02 ± 0.52^{bc}	10.56 ± 0.16^{b}	0.15 ± 0.02^{defg}	0.23 ± 0.23^{i}

Values are given as means ± SE (n = 3). Values followed by different letters in superscript within columns are significantly different at $p<0.05$.

Table 4.13: Mineral (Fe, Zn, Cu, Pb and Cd) composition (mg/100g dw) of red seaweeds along the Kenya coast.

Species	Fe	Zn	Cu	Pb	Cd
Acanthophora spicifera	432.64 ± 92.78^{cde}	27.77 ± 2.23^{bc}	21.43 ± 0.99^{b}	0.08 ± 0.01^{g}	2.92 ± 0.49^{cde}
Chondrophycus papillosus	172.54 ± 22.07^{ghij}	35.48 ± 4.36^{bc}	17.15 ± 2.88^{b}	0.10 ± 0.03^{efg}	2.33 ± 0.51^{cdefgh}
Eucheuma denticulatum	57.85 ± 19.46^{ij}	21.51 ± 4.64^{bc}	29.81 ± 4.76^{b}	0.09 ± 0.04^{fg}	0.83 ± 0.12^{fghi}
Gracilaria arcuata	132.06 ± 5.53^{ghij}	24.41 ± 0.19^{bc}	15.38 ± 0.29^{b}	0.18 ± 0.02^{cdefg}	0.31 ± 0.31^{hi}
Gracilaria salicornia	139.17 ± 20.58^{ghij}	50.05 ± 12.80^{abc}	12.20 ± 1.44^{b}	0.18 ± 0.02^{cdefg}	1.08 ± 0.20^{efghi}
Hypnea musciformis	120.89 ± 1.91^{hij}	36.85 ± 8.44^{bc}	16.11 ± 1.21^{b}	0.10 ± 0.03^{defg}	0.28 ± 0.41^{hi}
Hypnea sp.	138.58 ± 0.76^{ghij}	31.14 ± 0.38^{bc}	31.48 ± 0.33^{b}	0.05 ± 0.03^{g}	7.04 ± 0.40^{b}
Laurencia intermedia	81.18 ± 16.71^{ij}	32.93 ± 3.47^{bc}	15.59 ± 0.88^{b}	0.21 ± 0.07^{cdefg}	0.68 ± 0.11^{fghi}
Soliera robusta	243.26 ± 3.58^{fghi}	16.74 ± 0.73^{bc}	4.99 ± 0.27^{b}	0.03 ± 0.01^{g}	1.85 ± 0.21^{cdefghi}
Mean*	256.66 ± 23.85	34.84 ± 2.15	19.70 ± 2.81	0.20 ± 0.01	2.03 ± 0.15

* Values of means of the mineral elements (Tables 4.11 to 4.13).

Values are given as means ± SE (n = 3). Values followed by different letters in superscript within columns are significantly different at $p<0.05$.

The major mineral was iron with a mean value of 256.66 ± 23.85 mg/100g whereas the lowest was lead with a mean value of 0.20 ± 0.01 mg/100g ($p<0.05$) (Table 4.13). *Sargassum* sp. had the highest iron content (2226.91 ± 31.29 mg/100g; $p<0.05$) whereas *Cystoseira trinodis* had the lowest iron content (45.81 ± 0.74 mg/100g; $p<0.05$) (Table 4.12). The iron content of *Sargassum* sp. was significantly higher than those recorded for *S. cristaefolium* and *S. oligocystum* in this study, 25.82 mg/100g in *S. oligocystum* (Muraguri *et al.*, 2016) and 68.21 mg/100g obtained in *S. polycystum* (Matanjun *et al.*, 2009). The iron content of *Cystoseira trinodis* was higher than in *C. humilis* (17 mg/100g), *C. compressia* (14.97 mg/100g), *C. nodicaulis* (41.31mg/100g) and *C. baccata* (10.96 mg/100g) but lower than of *C. immariscifolia* (50.81 mg/100g) studied by Vizetto-Duarte *et al.* (2016).

Halimeda macroloba had the highest zinc content (81.95 ± 21.02 mg/100g; $p<0.05$) while *Chaetomorpha crassa* had the lowest zinc content (7.11 ± 0.25 mg/100g; $p<0.05$). The zinc content of *Halimeda macroloba* was considerably higher than 0.07 mg/100g found in *H. macroloba* studied by Anantharaman *et al.* (2010). The zinc content of *Chaetomorpha crassa* reported here was higher than in other studied chlorophytes such as 3.51 mg/100g in *Caulerpa lentifiera* (Matanjun *et al.*, 2009), 2.6 mg/100g in *Caulerpa lentifera* and 3.3 mg/100g in *Ulva reticulatum* (Ratana-arporn and Chirapart, 2006).

Ulva reticulatum had the highest copper content (106.41 ± 47.89 mg/100g; $p<0.05$) whereas *Sargassum cristaefolium* had the lowest copper content (4.05 ± 0.30 mg/100g; $p<0.05$). The copper content of *Ulva reticulatum* reported here was considerably higher than 1.45 ± 0.21 mg/100g in *Ulva lactuca* (Tabarsa *et al.*, 2012), 0.006 mg/100g in *Ulva reticulatum* (Ratana-arporn and Chirapart, 2006), 1.0 mg/100g in *Ulva pertusa* and 0.9 mg/100g in *Ulva intestinalis* (Benjama and Masniyom, 2011) and 0.17 mg/100g in *Ulva reticulatum* (Anantharaman *et al.*, 2010). In this study, the copper content of *Sargassum* sp. was higher than 0.03 mg/100g in *Sargassum polycystum* (Matanjun *et al.*, 2009).

Hydroclathrus clathrus had the highest lead (0.72 ± 0.00 mg/100g) and cadmium (10.21 ± 0.41 mg/100g) contents whereas *Dictyota* sp. 2 had the lowest lead (0.02 ± 0.00 mg/100g) and cadmium (0.20 ± 0.04 mg/100g) (p<0.05). The levels of cadmium and lead in the seaweeds collected were higher than the permissible limits of 2 mg/100g (CAC, 2003) and 0.5 mg/100g (Walker, 1988), respectively in foods. Therefore, little amounts of these seaweeds are recommended in food product development.

The mineral content of seaweed species varied with algal divisions as shown in Table 4.14. The chlorophytes had the highest magnesium content of 2052.42 ± 172.13 mg/100g while phaeophytes and rhodophytes had the lowest magnesium content of 1492.82 ± 71.84 and 1081.06 ± 54.35 mg/100g (p<0.05), respectively. The chlorophytes had the highest calcium content of 1357.05 ± 140.88 mg/100g while phaeophytes and rhodophytes had the lowest magnesium content of 693.32 ± 48.68 and 529.51 ± 74.07 mg/100g (p<0.05), respectively.

Rhodophyta and phaeophyta had the highest potassium content of 185.95 ± 33.63 and 169.62 ± 18.57 mg/100g, respectively while chlorophytes had the lowest potassium content of 41.47 ± 6.32 mg/100g (p<0.05) (Table 4.14). Similarly, various studies have reported high amounts of potassium in red and brown seaweeds than in green seaweeds (Larrea-Marín *et al.*, 2010; Desideri *et al.*, 2016; Neto *et al.*, 2018; Ometto *et al.*, 2018). The rhodophytes had the highest sodium content of 112.69 ± 12.00 mg/100g whereas phaeophytes and chlorophytes had the lowest sodium content of 82.02 ± 8.12 and 53.98 ± 4.95 mg/100g (p<0.05), respectively. Generally, sodium and potassium contents in chlorophytes especially in *Ulva* spp. tend to be lower than those found in rhodophytes and phaeophytes (Circuncisão *et al.*, 2018). Muthuraman and Ranganathan (2004) suggested that seaweeds accumulate high sodium content due to their polysaccharide content. In this study, the rhodophytes had highest nitrogen free extract (NFE) probably justifying the highest sodium content.

Table 4.14: Mineral composition (mg/100g dw) of seaweeds collected by algal division

Mineral	Algal divisions		
	Rhodophyta	Chlorophyta	Phaeophyta
Sodium	112.69 ± 12.00^{a}	53.98 ± 4.95^{c}	82.02 ± 8.12^{b}
Potassium	185.95 ± 33.63^{a}	41.47 ± 6.32^{b}	169.62 ± 18.57^{a}
Na/K ratio	0.96 ± 0.15^{b}	1.71 ± 0.14^{a}	0.79 ± 0.08^{b}
Calcium	529.51 ± 74.07^{b}	1357.05 ± 140.88^{a}	693.32 ± 48.68^{b}
Magnesium	1081.06 ± 54.35^{c}	2052.42 ± 172.13^{a}	1492.82 ± 71.84^{b}
Phosphorus	2.52 ± 0.11^{ab}	2.27 ± 0.17^{b}	2.70 ± 0.14^{a}
Iron	188.72 ± 20.71^{b}	180.09 ± 16.38^{b}	381.07 ± 56.00^{a}
Zinc	36.14 ± 3.62^{ab}	40.55 ± 5.54^{a}	28.99 ± 1.58^{b}
Copper	16.31 ± 1.05^{a}	26.82 ± 9.06^{a}	16.97 ± 1.68^{a}
Lead	0.13 ± 0.01^{b}	0.24 ± 0.02^{a}	0.25 ± 0.03^{a}
Cadmium	1.85 ± 0.25^{a}	2.05 ± 0.23^{a}	2.19 ± 0.30^{a}

Values are given as means ± SE (n = 3). Values followed by different letters in superscript within rows are significantly different at $p<0.05$.

The chlorophytes had the highest Na/K ratio of 1.71 ± 0.14, followed by rhodophytes (0.96 ± 0.15) and the lowest in phaeophytes (0.79 ± 0.08). In this study, the Na/K ratio of the seaweeds ranged from 0.04 (*Dictyota* sp. 1) to 4.93 (*Hypnea* sp.) ($p<0.05$) which was lower than those found in some food products, such as olives (43.6), sausages (4.9) and cheddar cheese (8.7) (Ruperez, 2002; Paiva *et al.*, 2016). The intake of sodium chloride and diets with high Na/K ratio has been associated with the incidence of hypertension (Insel *et al.*, 2007). From the nutrition point of view, consumption of phaeophytes could contribute to substantial intakes of potassium, in order to balance the dietary Na/K ratio consequently reducing cases of hypertension among the affected population.

The iron content of phaeophytes (381.07 ± 56.00 mg/100g) was the highest whereas the lowest values were obtained in rhodophytes (188.72 ± 20.71

mg/100g) and chlorophytes (180.09 ± 16.38 mg/100g) ($p<0.05$). This is contrary to mean values calculated from a summary of mineral composition of European macroalgae by Circuncisão *et al.* (2018) where the Chlorophyta had the highest iron content (150.9 mg/100g dw) followed by Phaeophyta (27.4 mg/100g) and lowest, the rhodophytes (25.4 mg/100g). The chlorophytes had the highest zinc content (40.55 ± 5.54 mg/100g) followed by rhodophytes (36.14 ± 3.62 mg/100g) and lowest, the phaeophytes (28.99 ± 1.58 mg/100g) ($p<0.05$). These variations could be influenced naturally by differences in salinity, temperature, pH, sunlight, nitrogen availability, as well by the structural features of the seaweed (Nisizawa *et al.*, 1987; Fleurence and Le Coeur, 1993; Mabeau and Fleurence, 1993). The phaeophytes and chlorophytes had the highest lead content of 0.25 ± 0.03 and 0.24 ± 0.02 mg/100g, respectively while rhodophytes had the lowest lead content of 0.13 ± 0.01 mg/100g ($p<0.05$). The concentration of heavy metals varies with structural composition of seaweed (Mabeau and Fleurence, 1993) which suggests variation in degree of metal accumulation (Ito and Hori, 1989). This probably explains the differences in lead contents among the algal divisions. There were no significant differences in cadmium and copper contents among the algal groups ($p>0.05$). The presence of lead and cadmium in seaweeds is probably as a result of pollution of seawaters by industrial wastes and household sewerage.

There was no significant difference in magnesium. calcium, copper and lead contents among the three sites ($p>0.05$) (Table 4.15). The highest potassium content was found in seaweeds collected in Kibuyuni (199.97 ± 30.08 mg/100g) whereas the lowest potassium content was found in seaweeds collected in Mtwapa (117.97 ± 13.72 mg/100g) and Mkomani (70.57 ± 7.21 mg/100g) ($p<0.05$). Seaweeds collected in Mtwapa had the highest sodium content of 121.71 ± 15.73 mg/100g while those in Kibuyuni and Mkomani had the lowest sodium content of 82.51 ± 8.03 and 66.52 ± 7.07 mg/100g ($p<0.05$), respectively. Seaweeds collected in Kibuyuni and Mkomani had the highest phosphorus content of 2.67 ± 0.14 and 2.64 ± 0.13 mg/100g, respectively whereas those in Mtwapa had the lowest phosphorus of 1.89 ± 0.11 mg/100g ($p<0.05$).

Table 4.15: Mineral composition (mg/100g dw) of seaweeds collected by site

Mineral	**Sites**		
	Mkomani	Kibuyuni	Mtwapa
Sodium	66.52 ± 7.07^{b}	82.51 ± 8.03^{b}	121.71 ± 15.73^{a}
Potassium	70.57 ± 7.21^{b}	199.97 ± 30.08^{a}	117.97 ± 13.72^{b}
Calcium	844.04 ± 87.28^{a}	822.75 ± 92.16^{a}	862.47 ± 138.55^{a}
Magnesium	1403.60 ± 95.68^{a}	1591.03 ± 113.37^{a}	1597.27 ± 138.50^{a}
Phosphorus	2.64 ± 0.13^{a}	2.67 ± 0.14^{a}	1.89 ± 0.11^{b}
Iron	173.84 ± 11.57^{b}	335.35 ± 49.82^{a}	229.14 ± 25.62^{ab}
Zinc	46.03 ± 3.89^{a}	29.62 ± 3.28^{b}	25.29 ± 0.93^{b}
Copper	27.11 ± 7.46^{a}	15.33 ± 1.16^{a}	15.62 ± 1.24^{a}
Lead	0.22 ± 0.02^{a}	0.18 ± 0.02^{a}	0.24 ± 0.04^{a}
Cadmium	1.13 ± 0.14^{b}	2.41 ± 0.26^{a}	2.90 ± 0.35^{a}

Values are given as means ± SE (n = 3). Values followed by different letters in superscript within rows are significantly different at $p<0.05$.

Seaweeds collected in Kibuyuni had the highest iron content of 335.35 ± 49.82 mg/100g while those in Mkomani had the lowest iron content of 173.84 ± 11.57 mg/100g ($p<0.05$). The highest zinc content was found in seaweeds collected in Mkomani (46.03 ± 3.89 mg/100g) whereas the lowest zinc content was found in seaweeds collected in Kibuyuni (29.62 ± 3.28 mg/100g) and Mtwapa (25.29 ± 0.93 mg/100g) ($p<0.05$). Seaweeds collected in Mtwapa and Kibuyuni had the highest cadmium contents of 2.90 ± 0.35 and 2.41 ± 0.26 mg/100g, respectively whereas those in Mkomani had the lowest cadmium content (1.13 ± 0.14 mg/100g) ($p<0.05$). The high levels of heavy metals in seaweeds could be attributed to the disposal of industrial wastes and untreated sewerage into the seawaters.

This study has revealed variations in mineral contents with species, algal divisions and sites. Different species of seaweeds collected from the same sites and month showed variations in chemical composition. For instance, the green seaweed

Caulerpa racemosa, the brown *Sargassum* sp. and the red *Acanthophora spicifera* collected at Mkomani in October 2013 had variations in chemical composition. This further suggests differences in chemical composition among algal division. Seaweed species of the same genus such as *Codium*, *Gracilaria* and *Dicytota* had significant variations in crude ash, crude fibre and crude protein, respectively. This explains the significant differences in the chemical composition of seaweed species of the same genus. In this study, the high mineral composition of the seaweeds definitely indicates the possible utilization as food supplements to improve the nutritive value of human diets.

4.4 Baked seaweed biscuits

4.4.1 Formulation of seaweed biscuits

Dictyota sp. 2 that had the lowest lead and cadmium contents and high levels of iron and zinc (See Table 4.12) was thus selected for incorporation into the biscuit as described previously in Section 3.6.

4.4.2 Physical characteristics of the wheat-seaweed biscuits

The results in Table 4.16 showed that the width, thickness and spread factor of the biscuits varied significantly with seaweed content ($p<0.05$). There was a general increase in width and decrease in thickness of the biscuits with seaweed content. This was probably due to a decrease in dough viscosity with increased seaweed content and decreased wheat flour. Similar studies by Hoseney and Rogers (1994) and Chauhan *et al.* (2016) showed an increase in width with the incorporation of non-gluten ingredients in wheat-baked products. Kumar *et al*. (2018) reported that with increased *Caulerpa racemosa* levels, the thickness and diameter of biscuits reduced. This was attributed to dilution effect on the gluten (Ajila *et al.*, 2008) due to addition of seaweed which in turn affected the formation of suitable dough.

Table 4.16: Physical properties of baked seaweed biscuits

Seaweed content (%)	Biscuit width, *W* (mm)	Biscuit thickness, *T* (mm)	Spread factor (10**W/T*)
0	63.72 ± 0.01^{e}	7.61 ± 0.01^{a}	83.72 ± 0.07^{e}
1	63.76 ± 0.01^{d}	7.61 ± 0.01^{a}	83.73 ± 0.05^{e}
2	63.78 ± 0.01^{d}	7.58 ± 0.00^{b}	84.11 ± 0.01^{d}
3	63.88 ± 0.01^{c}	7.56 ± 0.01^{c}	84.48 ± 0.06^{c}
4	64.12 ± 0.01^{b}	7.47 ± 0.01^{d}	85.41 ± 0.06^{b}
5	64.28 ± 0.01^{a}	7.44 ± 0.01^{e}	86.42 ± 0.05^{a}

Values are given as means ± SE (n = 6). Values followed by different letters in superscript within columns are significantly different at $p<0.05$.

There was no significant difference ($p>0.05$) in the spread factor between biscuits with 0 and 1% seaweed content. Biscuits with 2, 3, 4 and 5% seaweed had significant differences in spread factor ($p<0.05$). The spread factor is an important quality parameters of biscuits. It is, however, a relatively complex phenomenon which ultimately contributes to bite, texture, overall acceptability and grain finesse of biscuits (Bose and Shams-ud-din, 2010). The two main factors affecting the spread factor are the dough expansion by gravitational flow and leavening. Generally, higher spread factor is prefered for biscuits (Kumar *et al*., 2018). The biscuits with the 0 and 1% seaweed content had the highest thickness compared to the others due to adequate expansion of dough by leavening. The increasing spread factor with seaweed content can be attributed to dilution of water-absorbing constituents like protein and carbohydrates. The seaweed incorporated in the biscuits, *Dictyota* sp. 2, had the lowest protein (Mwalugha *et al.*, 2015). Patel and Rao (1996) and Hooda and Jood (2005) observed a reduction in spread factor of biscuits with substitution of wheat flour for high protein and/or high fibre non-gluten flours.

4.4.3 Sensory attributes of the seaweed biscuits

The sensory attributes of baked biscuits are presented in Figure 4.3.

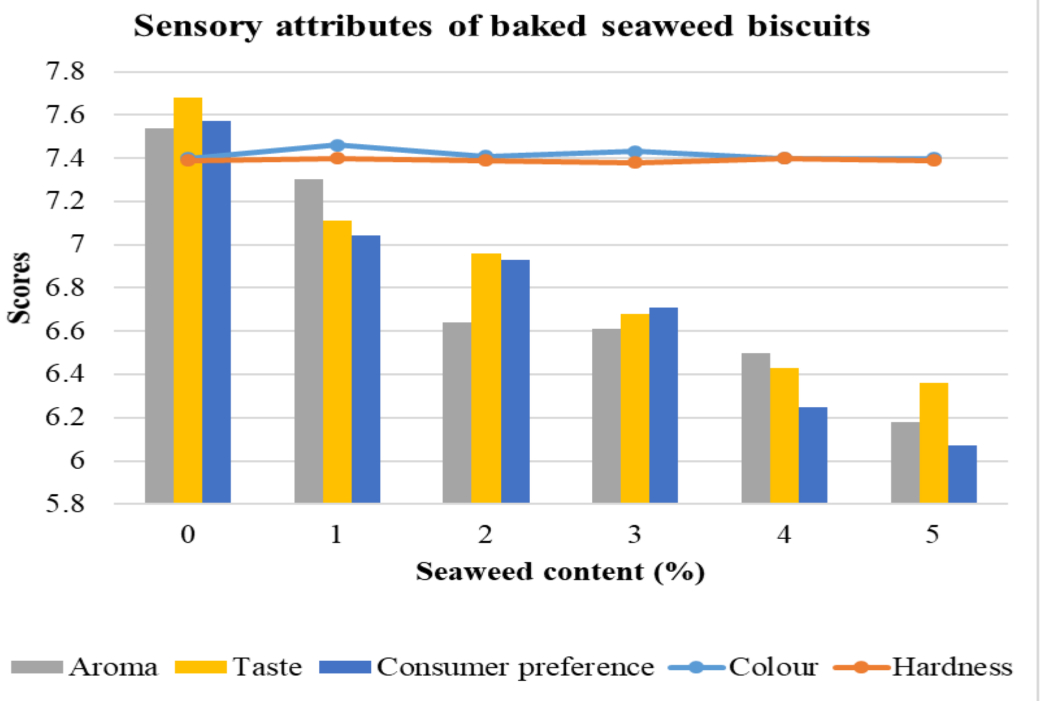


Figure 4.3: Sensory attributes of the baked seaweed biscuits.

According to the results presented in Figure 4.3, there were no significant changes observed in colour (Plate 4.4) and the hardness of the baked biscuits (*p*>0.05).

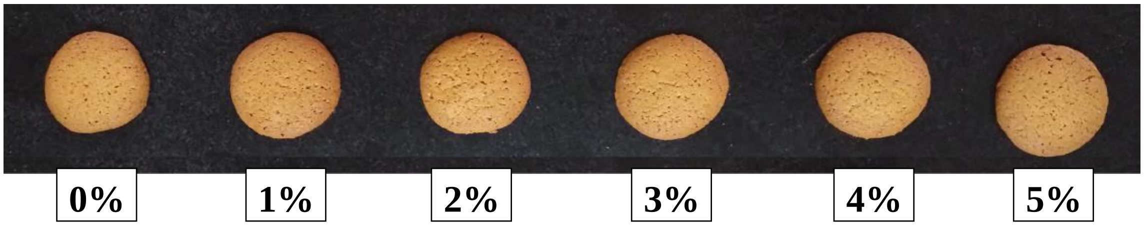


Plate 4.4: Color of baked biscuits of different seaweed (*Dictyota* sp. 2) concentration.

During the baking process, colour was promoted by the Maillard reaction between reducing sugars and protein, and starch caramelization and dextrinization which are accelerated by heating (Cheng and Bhat, 2016). *Dictyota* sp. 2 is a seaweed with low crude protein but relatively high carbohydrates (Mwalugha *et al.*, 2015). Its incorporation into the biscuits in smaller amounts probably did not significantly change the protein-sugar balance in the dough mixture hence did not affect the browning reactions. Other factors such as type of amino compounds, sugars, temperature, water activity and pH affect the browning reactions (Jan *et al.*, 2016).

Eating quality of biscuits is directly related to hardness of biscuits. Hardness, considered an important parameter, measures the peak force required to snap a biscuit. The hardness of biscuits is correlated to the protein content of the flour due to interactions during dough development (Cheng and Bhat, 2016). In this study, the incorporation of seaweed (up to 5%) in the biscuits did not significantly affect the scores in hardness. There was probably little or no significant interference in total protein content due to the little amount of seaweeds incorporated.

There was a significant decrease in scores for aroma, taste and consumer acceptability with seaweed content. This was probably due to the increase in the characteristic fishy odour of the seaweed in the biscuits. Heat treatment is one of the most common method in food processing aimed at improving the palatability, digestibility and shelf-life of foods (Murcia *et al.*, 2002; Zhang and Hamauzu, 2004; Borowski *et al.*, 2015) while altering the physical and physicochemical characteristics of foods. These alterations result in loss of nutritional and organoleptic properties (Vallejo *et al.*, 2002; Zhang and Hamauzu, 2004; Borowski *et al.*, 2015). During the baking process, the Maillard reactions between the reducing sugars with amines from the seaweed (that impart the fishy smell) resulted in off-flavours that were undesirable. Despite the significant decrease in aroma, taste and consumer preference with increase in seaweed content (p<0.05), the scores for the seaweed biscuits (1–5%) were only slightly lower than the control (0% seaweed), and all the seaweed biscuits were at acceptable values suggesting potential incorporation of seaweeds in biscuits.

4.4.4 Iron and zinc levels of the developed product

The iron and zinc levels of the seaweed biscuits were evaluated and the results showed a significant increase in iron and zinc levels with seaweed content ($p<0.05$) (Table 4.17). The biscuits with 1–5% seaweed content were within permissible limits for lead (CAC, 2003). However, the biscuits with 4 and 5% seaweed content had cadmium contents above the permissible limits in foods (Walker, 1988). The recommended maximum amount of seaweed (*Dictyota* sp. 2) content in biscuits was 3%. The biscuits with 1, 2 and 3% seaweed content provided RDI values for iron at 30.58, 47.25 and 63.67%, respectively for male (National Research Council, 1989). The RDI values for zinc in 1, 2 and 3% seaweed content were 20.13, 23.47 and 25.73%, respectively for male (National Research Council, 1989).

Table 4.17: Iron and zinc levels (mg/100g) of baked seaweed biscuits

Seaweed content (%)	**Iron^1 (mg/100g)**	**Zinc^2 (mg/100g)**	**Cadmium^3 (ppb)**	**Lead^3 (ppb)**
0	1.21 ± 0.03^f	2.66 ± 0.04^f	-	-
1	3.67 ± 0.05^e	3.02 ± 0.05^e	13.89	1.39
2	5.67 ± 0.07^d	3.52 ± 0.04^d	27.78	2.78
3	7.64 ± 0.10^c	3.86 ± 0.02^c	41.67	4.17
4	9.96 ± 0.05^b	4.19 ± 0.02^b	55.56	5.56
5	12.18 ± 0.04^a	4.54 ± 0.04^a	69.44	6.94
Limits (ppb)	-	-	^{4}50	^{5}200

Values for iron and zinc are given as means ± SE (n = 6). Values followed by different letters in superscript within columns are significantly different at $p<0.05$.

[1] RDI for iron for male: 12 mg (National Research Council, 1989)

[2] RDI for zinc for male: 15 mg (National Research Council, 1989)

[3] Values calculated from Cd and Pb levels of *Dictyota* sp. 2 (Table 4.14)

[4] Source: Walker, 1988

[5] Source: CAC, 2003

Despite the biscuits with 1–3% seaweed content being within the permissible limits for lead and cadmium, the consumption of more than 100g daily introduces significant amounts of cadmium to the human body within a short period of time; as cadmium accumulates in the bones, liver and kidney, and results in adverse effects (Jaishankar *et al.*, 2014).

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

This research study revealed that there was a wide variety of seaweeds identified among the three sites (Mkomani, Kibuyuni and Mtwapa) along the Kenya coast. However, some of the seaweeds species identified such as *Hypnea* spp., *Codium* spp., *Caulerpa* spp., *Cystoseira* spp., *Dictyota* spp., *Enteromorpha* spp., *Eucheuma denticulatum*, *Gracilaria* spp. and *Laurencia intermedia* were only available in specific sites and months.

The seaweeds collected along the Kenya coast were a good source of dietary fibre, proteins, carbohydrates and minerals. There was variation in the nutrient composition of seaweeds with species, sites and seasons. The seaweeds from Chlorophyta and Rhodophyta classes were the most nutritionally rich with respect to crude protein, crude fibre and nitrogen-free extract (NFE). The highest crude protein, crude fibre and NFE were found in *Hypnea* species, *Laurencia intermedia* and *Dictyota* species, respectively. The highest crude fat and crude ash were found in *Dictyota* species and *Codium* species, respectively.

The major minerals were magnesium and calcium. The species in the Chlorophyta class had the highest magnesium and calcium contents. The calcium contents of most of these seaweeds were higher than in common household vegetables such as cabbages, lettuce and spinach thus could be potential food supplements in the human diet. The Na/K ratio was lowest in phaeophytes thus could be a potential source of potassium to balance the dietary Na/K ratio associated with intake of sodium chloride and foods with high Na/K ratio (such as cheese and sausages) to avoid cases of hypertension. The brown, red and green seaweeds were rich in iron, zinc and copper above the recommended daily intake thus could be utilized in small proportions for recommended daily intake when incorporated in foods.

The research confirmed that seaweeds containing high mineral composition may be incorporated in common food vehicles such as biscuits through value addition to avail vital nutrient especially those of public health concern in Kenya such as iron and zinc. The recommended levels of seaweed (*Dictyota* sp. 2) in biscuits was 3%. Consumption of 100g of the seaweed biscuits would contribute significantly to the recommended daily intake levels for iron and zinc to combat deficiencies in iron and zinc among affected populations. Although the biscuits with 1–3% seaweed content had levels of cadmium and lead within permissible limits and were of high consumer acceptability, there is a concern of cadmium accumulation in human beings over time.

In general, the hypotheses that guided the study were supported by the findings of this research study. The chemical composition of seaweeds varied with species, site and season. Kenyan seaweeds were found to be rich sources of essential nutrients and could be utilized in developing a new segment of functional foods – composite seaweed food products.

5.2 Recommendations

Based on the findings of this research, the following recommendations are made:

1. There is need to identify seaweeds in other sites along the Kenya coast and assess their nutritional potential in developing seaweed-based products.
2. There is need to conduct further chemical analysis of seaweeds in Kenya to understand the high crude ash contents for prior to utilization in food production.
3. Further research should be conducted to characterize the carbohydrates in seaweeds from the Kenya coast for their utilization in the industrial market.

REFERENCES

AACC. (1995). *Approved methods of the American Association of Cereal Chemists*. *Official methods of analysis* (9th Ed.). St Paul, MN: American Association of Cereal Chemists.

Abreu, M. H., Pereira, R., Yarish, C., Buschmann, A. H. and Sousa-Pinto, I. (2011). IMTA with *Gracilaria vermiculophylla*: Productivity and nutrient removal performance of the seaweed in a Land-based pilot scale system. *Aquaculture, 312* (1-4), 77-87.

Adeola, A. A. and Ohizua, E. R. (2018). Physical, chemical, and sensory properties of biscuits prepared from flour blends of unripe cooking banana, pigeon pea, and sweet potato. *Food Science and Nutrition, 6*(3), 532–540.

Adeyeye, S. A. and Akingbala, J. O., (2015). Physico-chemical and functional properties of cookies produced from sweet potato-maize flour blends. *Food Science and Quality Management*, *43*, 48–57.

Ainis, A., Vellanoweth, R., Lapeña, Q. and Thornber, C. S. (2014). Using non-dietary gastropods in coastal shell middens to infer kelp and seagrass harvesting and paleoenvironmental conditions. *Journal of Archaeological Science, 49*, 343–360.

Alarif, W., Abou-Elnaga, Z., Ayyad, Z. and Al-Lihaibi, S. (2010). Insecticidal metabolites from the green alga *Caulerpa racemosa*. *Clean – Soil, Air, Water*, *38*, 548–557.

Anantharaman, P., Karthikaidevi, G., Manivannan, K., Thirumaran, G. and Balasubramanian, T. (2010). Mineral composition of marine macroalgae from Mandapam coastal regions; southeast coast of India. *Recent Research Science and Technology, 2*(10), 66-71.

Anantharaman, P., Parthiban, C., Saranya, C., Girija, K., Hemalatha, A. and Suresh, M. (2013). Biochemical composition of some selected seaweeds from Tuticorian coast. *Advances in Applied Science Research, 4*(3), 362-366.

Anderson, J., Jackson, A. J., Matty, A. J. and Capper, B. S. (1984). Effects of dietary carbohydrate and fibre on the tilapia *Oreochromis niloticus* (Linn.). *Aquaculture, 37*(4), 303-314.

Angstwurm, K. (1995). Fucoidan, a polysaccharide inhibiting leukocyte rolling, attenuates inflammatory responses in experimental pneumococcal meningitis in rats. *Neuroscience Letters, 191*, 1-4.

Awang, H., Daud, Z. and Hatta, M. Z. M. (2015). Hydrology properties and water quality assessment of the Sembrong Dam, Johor, Malaysia. *Procedia-Social Behavoural Science*, *195*, 2868–2873.

AOAC. (2005). *Association of Officiating Analytical Chemists*. *Official method of Analysis* (18th Ed.). Washington DC: Association of Officiating Analytical Chemists.

Arasaki, S. and Arasaki, T. (1983). *Low Calorie, High Nutrition Vegetables from the Sea to Help You Look and Feel Better*. Tokyo, Japan: Japan Publications.

Arori, M. K., Muthumbi, A. W. N., Mutia, G. M. and Nyonje, B. (2019). Potential of seaweeds (*Hypnea cornuta* and *Hypnea musciformis*) in Nile tilapia (*Oreochromis niloticus*) fingerlings diets. *International Journal of Fisheries and Aquatic Studies*, *7*(2), 103-107.

Aroyehun, A. Q., Palaniveloo, K., Ghazali, F., Rizman-Idid, M. and Razak, S. A. (2019). Effects of seasonal variability on the physicochemical, biochemical and nutritional composition of Western Penisular Malaysia *Gracilaria manilaensis*. *Molecules, 24*, 3298-3331.

Baharim, N. B., Yusop, Z., Yuso, I., Tahir, W., Askari, M., Othman, Z. and Abidin, M. R. Z. (2016). The relationship between heavy metals and trophic properties in Sembrong Lake, Johor. *Sains Malaysia*, *45*, 43–53.

Balaji, N. (1991). *Modelling of transient temperature distribution during bread baking by finite difference analysis*. B. Tech thesis, India: Indian Institute of Technology.

Baltasvias, A., Jurgens, A. and Van Vliet, T. (1999). Fracture properties of short-dough biscuits: Effect of composition. *Journal of Cereal Science*, *29*, 235-244.

Banerjee, K., Ghosh, R., Homechaudhuri, S. and Mitra, A. (2009). Seasonal variation in the biochemical composition of red seaweed *Ctanella repens* from Gangetic delta, northeast coast of India, *Journal of Earth System Science*, *118*(5), 497-505.

Benjama, O. and Masniyom, P. (2011). Nutritional composition and physicochemical properties of two green seaweeds (*Ulva pertusa* and *U. intestinalis*) from the Pattani Bay in Southern Thailand. *Songklanakarin Journal of Science and Technology*, *33*(5), 575-583.

Benjama, O. and Masyonim, P. (2012). Biochemical composition and physicochemical properties of two red seaweeds (*Gracilaria fisheri* and *G. tenuistipitata*) from the Pattani Bay in Southern Thailand. *Songklanakarin Journal of Science and Technology*. *34*(2), 223-230.

Bird, K. T., Millamena, O. M. and Kanazawa, A. (1990). Use of kappa-carrageenan micro bound diet (C-MBD) as feed for *Penaeus monodon* larva. *Marine Biology*, *193*, 169-173.

Bolton, J. J., De Clerck, O. and John, D. M. (2003). Seaweed diversity patterns in Sub-Saharan Africa. In Decker, C., Griffiths, C.L., Prochazka, K., Ras, C., Whitfield, A. (Eds.), *Proceedings of the Marine Biodiversity in Sub-*

Saharan Africa: The Known and the Unknown. Cape Town, South Africa, 23rd – 26th September 2003, 229–241 (CD-ROM format).

Bolton, J. J., Oyieke, H. A. and Gwada, P. (2007). The seaweeds of Kenya: Checklist, history of seaweed study, coastal environment, and analysis of seaweed diversity and biogeography. *South African Journal of Botany, 73*, 76–88.

Bolton, J. J., Robertson-Andersson, D. V., Shuuluka, D. and Kandjengo, L. (2009). Growing *Ulva* (Chlorophyta) in integrated systems as a commercial crop for abalone feed in South Africa: a SWOT analysis. *Journal of Applied Phycology*, *21*, 575–583.

Borowski, J., Narwojsz, J., Borowska, E. J. and Majewska, K. (2015). The effect of thermal processing on sensory properties, texture attributes, and pectic changes in broccoli. *Czech Journal of Food Sciences*, *33,* 254–260.

Bose, D. and Shams-ud-din, S. (2010). The effect of chickpea (*Cicer arietinim*) husk on the properties of cracker biscuits. *Journal of Bangladesh Agricultural University, 8*(1), 147–152.

Botana, L. (2007). *Phycotoxins: Chemistry and Biochemistry* (2nd Ed.). Iowa: Blackwell Publishing, pp 11-57.

Boussiba, S. and Richmond, A. E. (1979). Isolation and characterization of phycocyanins from the bluegreen alga *Spirulina platensis*. *Archives of Microbiology, 120*, 155-159.

Brabin, B. J., Hakimi, M. and Pelletier, D. (2001a). An analysis of anaemia and pregnancy-related maternal mortality. *Journal of Nutrition*, *131*(2), 604–614.

Brabin, B. J., Premji, Z. and Verhoeff, F. (2001b). An analysis of anaemia and child mortality. *Journal of Nutrition*, *131*(2), 636–645.

Brandon, E. F., Janssen, P.J. and de Wit-Bos, L. (2014). Arsenic: Bioaccessibility from seaweed and rice, dietary exposure calculations and risk assessment. *Food Additives and Contaminants, 31*, 1993–2003.

Briggs, M. R. P. and Smith, S. J. F. (1993). Macroalgae in aquaculture: An overview and their possible roles in shrimp culture. *Proceedings of a conference on marine biotechnology in the Asia Pacific. Bangkok, Thailand*, 137–143.

Brown, E. M., Allsopp, P. J., Magee, P.J., Gill, C. I. R., Nitecki, S., Strain, C. R. and McSorley, E. M. (2014). Seaweed and human health. *Nutrition Review, 72*, 205–216.

Brownlee, I. A, Fairclough, A. C., Hall, A. C. and Paxman, J. R. (2011). Dietary seaweed and human health. Bournemouth University UK, *Bournemouth University International Centre for Tourism and Hospitality Research*, 82-88.

Burtin P. (2003). Nutritional value of seaweeds. *Electronic Journal of Environmental, Agricultural and Food Chemistry, 2*(4), 498-503.

Caballero, B., Allen, L. and Prentice, A. (2005). *Encyclopaedia of Human Nutrition* (2nd Ed.). Oxford: Elsevier Ltd.

Camberlin, P. and Philippon, N. (2002). The East African March-May rainy season: associated atmospheric dynamics and predictability over the 1968-97 period. *Journal of Climate, 15*, 1002-1019.

Chakraborty, S. and Santra, S. C. (2008). Biochemical composition of eight benthic algae collected from Sunderban. *Indian Journal of Marine Science, 37*, 329–332.

Chan, J. C. C., Cheung, P. C. and Ang Jr, P. O. (1997). Comparative studies on the effect of three drying methods on the nutritional composition of seaweed *Sargassum hemiphyllum* (Turn.). *Journal of Agriculture and Food Chemistry, 45*, 3056–3059.

Chapman, V. J. and Chapman, D. J. (1980). *Seaweeds and their uses* (3rd Ed.). London: Chapman and Hall.

Chapman, A. S., Stévant, P. and Larssen W. E. (2015). Food or fad? Challenges and opportunities for including seaweeds in a Nordic diet. *Botanica Marina*, *58*, 423–433.

Charreau, B. (1997). Efficiency of fucans in protecting porcine endothelial cells against complement activation and lysis by human serum. *Transplantation Proceedings*, *29*, 889-890.

Chauhan, A., Saxena, D. C. and Singh, S. (2016). Physical, textural and sensory characteristics of wheat and amaranth flour blend cookies. *Cogent Food and Agriculture*, *2*, 1-8.

Chavan, J. K. and Kadam, S. S. (1993). Nutritional enrichment of bakery products by supplementation with non-wheat flours. *Critical Reviews in Food Science and Nutrition*, *33*(3), 189-226.

Cheng, Y. F. and Bhat, R. (2016). Functional, physicochemical and sensory properties of novel cookies produced by utilizing underutilized jering (*Pithecellobium jiringa* Jack.) legume flour. *Food Bioscience, 14*, 54–61.

Chevallier, S., Colonna, P. A., Della Valle, G. and Lourdin, D. (2000). Contribution of major ingredients during baking of biscuit dough systems. *Journal of Cereal Science*, *3*, 241-252.

Chevallier, S., Della Valle, G., Colona, P., Broyart, B. and Trystram, G. (2002). Structural and chemical modifications of short batter during baking. *Journal of Cereal Science*, *35*, 1-10.

Chinma, C. E., James, S., Imam, H., Ocheme, O. B., Anuonye, J. C. and Yakubu, C. M. (2011). Physicochemical and sensory properties and *in-vitro* digestibility of biscuits made from blends of tigernut (*Cyperus esculentus*) and pigeon pea (*Cajanus cajan*). *Nigerian Journal of Nutritional Sciences*, *32*, 55–62.

Chopin, T. (2012). Aquaculture, Integrated Multi-Trophic (IMTA), pp. 542-564. In: Meyers, R.A. (Ed.), *Encyclopedia of Sustainability Science and Technology*. Dordrecht, Netherlands: Springer.

Circuncisão, A. R., Catarino, M. D., Cardoso, S. M. and Silva, A. M. S. (2018). Minerals from Macroalgae Origin: Health Benefits and Risks for Consumers. *Marine Drugs*, *16*(11), 1-30.

Codex Alimentarius Commission (CAC) (2003). Evaluation of certain food additives and contaminants. Rome: FAO/WHO. Retrieved from http://www.fao.org/3/i3243e/i3243e.pdf

Combet, E. Jarlot, A., Aidoo, K. E. and Lean, M. E. J. (2013). Development of a nutritionally balanced pizza as a functional meal designed to meet published dietary guidelines. *Public Health Nutrition*, *17*, 2577–2586.

Coppejans, E., De Clerck, O., Leliaert, F. and Dargent, O. (2001). Progress of the taxonomic research on the macroalgae (Chlorophyta, Phaeophyta, Rhodophyta) along the East African coast. In: Richmond, M. D., Francis, J. (Eds.), Marine Science Development in Tanzania and Eastern Africa. *Proceedings of the 20th Anniversary Conference on Advances in Marine Science in Tanzania*, pp 401-418.

Cousins, R. I. (1996). Zinc. In: Present knowledge in nutrition. (Eds.), Zeigler, E. E., Filer, L. J. Washington DC: ILSI Press.

Cronin, K. and Preis, C. (2000). A statistical analysis of biscuit physical properties as affected by baking. *Journal of Food Engineering*, *46*, 217-225.

Das, A. (1990). Metal ion-induced toxicity and detoxification by chelation therapy. *A textbook on medical aspects of bioinorganic chemistry*. Delhi, India: CBS Press, pp. 17-58.

Dave, M. J., Parekh, R. G., Gang, S. K. and Metha, D. J. (1979). Preparation of seaweed meal for the feeding of farm animals. *Research Indicators, 15*(2), 34-38.

Davis, T. A., Volesky, B. and Mucci, A. (2003). A review of the biochemistry of heavy metal biosorption by brown algae. *Water Research, 37*, 4311–30.

Dawczynski, C., Schubert, R. and Jahreis, G. (2007). Amino acids, fatty acids, and dietary fibre in edible seaweed products. *Food Chemistry, 103*(3), 891-899.

Dawes, C. J. (1998). *Marine botany* (2nd Ed.). New York NY: John Wiley and Sons Inc.

Dawes, C. J., Lawrence, J. M. and Cheney, D. P. (1974). Ecological studies of Floridian *Eucheuma* (Rhodophyta, Gigartinales) III. Seasonal variation of carrageenan, total carbohydrate, protein and lipid. *Bulletin of Marine Science*, *24*, 287-299.

de la Torre-Castro, M. and Rönnbäck, P. (2004). Links between humans and seagrasses - An example from Tropical East Africa. *Ocean Coastal Management*, *47*, 361–387

Delaney, A., Frangoudes, K. and Li, S. A. (2016). *Society and seaweed: understanding the past and present*. In Seaweed in Health and Disease Prevention (Fleurence, J. & Levine, I. editors), London: Elsevier Academic Press, pp. 7–40.

Desideri, D., Cantaluppi, C., Ceccotto, F., Meli, M. A., Roselli, C. and Feduzi, L. (2016). Essential and toxic elements in seaweeds for human consumption. *Journal of Toxicology and Environmental Health*, *79*, 112–122

Dettmar, P. W., Strugala, V. and Craig-Richardson, J. (2011). The key role alginates play in health. *Food Hydrocolloids*, *25*, 263–266.

Dhargalkar, V. K., Jagtap, T. G. and Untawale, A. G., (1980). Marine macroalgae of Orissa, East coast of India. *Indian Journal of Marine Sciences*, *9*(4), 297-299.

Dillehay, T. T. D., Ramirez, C., Pino, M., Collins, M. B., Rossen, J. and Pino-Navarro, J. D. (2008). Monte Verde: Seaweed, Food, Medicine, and the People of South America. *Science*, *320*, 784–786.

Eklöf, J., Msuya, F., Lyimo, T. and Buriyo, A., (2012). Seaweed Farming in Chwaka Bay: A Sustainable Alternative in Aquaculture? In de la Torre-Castro, M and T. J Lyimo. People, Nature and Research in Chwaka Bay, Zanzibar town: WIOMSA, pp. 213–233.

Erlandson, J., Braje, T., Gill, K. and Graham, M. H. (2015). Ecology of the kelp highway: didmarine resources facilitate human dispersal from Northeast Asia to the Americas? *Journal of Island and Coastal Archaeology*, *10*, 392–411.

Fan-jie, Z., Zi-xuan, Y. and Li-jin, J. (1984). Isolation and characterization of R-phycocyanin from *Polysiphonia urceolata*. *Hydrobiologia*, *116/117*, 594-596.

FAO (2016). The State of World Fisheries and Aquaculture 2016 (SOFIA). *Contributing to food security and nutrition for all*. Rome: Food and Agriculture Organization.

Fernández-Ginés, J. M., Fernández-López, J., Sayas-Barberá, E. and Pérez-Alvarez, J. A. (2005). Meat Products as Functional Foods. *Journal of Food Science, 70*(2), 37-43.

Fleurence, J. (1999). Seaweed proteins: biochemical, nutritional aspects and potential uses. *Trends in Food Science and Technology, 10*, 25-28.

Fleurence, J. and Le Coeur, C. (1993). Influence of mineralization methods on the determination of the mineral content of brown seaweed *Undaria pinnatifida* by atomic absorption spectrophotometry. *Hydrobiologia, 260/261*, 531–4.

Fleurence, J., Morançais, M., Dumay, J., Decottignies, P., Turpin, V., Munier, M., Garcia Bueno, N. and Jaouen, P. (2012). What are the prospects for using seaweed in human nutrition and for marine animals raised through aquaculture? *Trends in Food Science and Technology, 27*, 57-61.

Foote, E. M., Sullivan, K. M., Ruth, L. J., Oremo, J., Sadumah, I., Williams, T. N. and Suchdev, P. S. (2013). Determinants of anemia among preschool children in rural, Western Kenya. *American Journal of Tropical Medicine Hygiene*, 3; *88*(4), 757–764.

Fredrick, F. and Mutheu, D. (2018). Lack of market keeping Kwale farmers from seaweed millions. Retrieved from https://www.businessdailyafrica.com/corporate/enterprise/Lack-of-market-keeping-Kwale-farmers-from-seaweed-millions/4003126-4359214-3flj

Friel, J. K. andrews, W. L. and Matthew, J. D. (1993) Zinc supplementation in very-low-birth-weight infants. *Journal of Pediatric Gastroenterology and Nutrition, 17*, 97–104.

Frikha, F., Kammoun, M., Hammami, N., Mchirgui, R. A., Belbahri, L., Gargouri, Y., Miled, N. and Ben-Rebah, F. (2011). Chemical composition and some biological activities of marine algae collected in Tunisia. *Ciencias Marinas*, *37*(2), 113–124.

Fusetani, N. (1987). Marine metabolites which inhibit development of Echinoderm embryos. In: Sheuer, P. J. (eds). Bioorganic Marine Chemistry. *Bioorganic Marine Chemistry*, *1*, 61-92.

Fusetani, N. and Hashimoto, K. (1984). Prostaglandin E2: a candidate for causative agent of "orgonori" poisoning. *Bulletin of the Chemical Society of Japan*, *50*, 465–469.

Fustier, P., Castaigne, F., Turgeon, S. L. and Biliaderis, C. G. (2009). Impact of endogenous constituents from different flour milling streams on dough rheology and semi-sweet biscuit making potential by partial substitution of a commercial soft wheat flour. *Swiss Society of Food Science and Technology*, *42*(1), 363–371.

Gaines, C. S. (1993). Collaborative studies on baking quality of cookies flour by wire-cut formulation. *Cereal Foods World*, *38,* 26–30.

Garrow, J. S., James, W. P. T. and Ralph, A. (1997). *Human Nutrition and Dietetics* (8th Ed.). London: Churchill Livingstone.

Gikwa, J. N. (2019). Prevalence of dietary supplements use and dietary practices among teachers in public secondary schools in Kikuyu, Kiambu County, Kenya. MSc thesis, Kenya: Kenyatta University.

Gomez-Pinchetti, J. L. and Martel-Quintana, A. (2016). Algae production and their potential contribution to a nutritional sustainability. *Journal of Environment and Health Science*, *2,* pp 1–3.

Gonzalez, R., Rodriguez, S., Romay, C., Ancheta, O., Gonzalez, A., Armesta, J., Remirez, D. and Merino, N. (1999). Anti-inflammatory activity of phycocyanin extract in acetic acid-induced colitis in rats. *Pharmacological Research*, *39*(1), 55-59.

Government of Kenya. (1999). Anaemia and status of iron, vitamin A and zinc in Kenya. *The 1999 National Survey Report*. UNICEF, Nairobi, Kenya.

Government of Kenya. (2009). *Food security in Kenya*. Ministry of Agriculture, pp. 1-31.

Government of Kenya. (2011). *Kenya National Micronutrient Survey*. Ministry of Health, pp. 12-122.

Gregson, R., Marwood, J. and Wuinn, R. (1979). The occurrence of prostaglandins PGE2 and PGF2a in a plant – the red alga *Gracilaria lichenoides*. *Tetrahedron Letters*, *46*, 4505–4506.

Gressler, V., Fujii, M. T., Martins, A. P., Colepicolo, P., Mancini, J. and Pinto, E. (2010). Biochemical composition of two red seaweed species grown on the Brazilian coast. *Journal of the Science of Food and Agriculture, 91*(9), 1687-1692.

Harlin, M. M. (1996). Allelochemistry in marine macroalgae. *Critical Reviews in Plant Sciences*, *5*, 237-249.

Hassan, S., Badr, I. H. A., Abd-Rabboh, H. and Meyerhoff, M. E. (2002). Determination of Carrageenan in Food Products Using Potentiometric Polyion Sensor. *Electroanalysis*, *14*(6), pp. 439-444.

Haugan, J. A. and Liaaen-Jensen, S. (1994). Algal carotenoids of brown algae (Phaeophycae). *Biochemical Systematics and Ecology*, *22*(1), 31-41.

Hay, M. E. (1996). The ecology and evolution of seaweed-herbivore interactions on coral reefs. *Coral Reefs*, *16*, 67-76.

Hesse, G. W. (1979). Chronic zinc deficiency alters neuronal function of hippocampal mossy fibers. *Science*, *205*, 1005–1007.

Hooda, J. and Jood, S. (2005). Organoleptic and nutritional evaluation of wheat biscuits supplemented with untreated and treated fenugreek flour. *Food Chemistry, 90*, 427–435.

Hoseney, R. C. and Rogers, D. E. (1994). Mechanism of sugar functionality in cookies. In H. Faridi (Ed.), *The science of cookie and cracker production* (pp. 203–226). New York, NY: Avi.

Hotz, C. and Brown, K. H. (2004). Assessment of the risk of zinc deficiency in populations and options for its control. *Food and Nutrition Bulletin*, *25*, 99-113.

Hurtado-Ponce, A. Q. (1995). Carrageenan properties and proximate composition of three morphotypes of *Kappaphycus alvarezii* Doty (Gigartinales, Rhodophyta) grown at two depths. *Botanica Marina*, *38*, 215-219.

Ihekoronye, A. I. and Ngoddy, P. O. (1985). *Integrated Food Science and Technology for the Tropics*. London: Macmillan press Ltd.

Insel, P., Ross, D., McMahon, K. and Bernstein, M. (2007). *Nutrition* (3rd ed.). Sudbury: Jones and Bartlett Publishers.

Ito, K. and Hori, K. (1989). Seaweed: chemical composition and potential uses. *Food Review International*, *5*, 101–144.

Jaishankar, M., Tseten, T., Anbalagan, N. and Beeregowda, K. (2014). Toxicity, mechanism and health effects of some heavy metals. *Interdisciplinary Toxicology*, *7*(2), 60-72.

James, C. S. (1996). *Analytical chemistry of foods*. New York, NY: Chapman and Hall.

Jan, R., Saxena, D. and Singh, S. (2016) Physico-chemical, textural, sensory and antioxidant characteristics of gluten-free cookies made from raw and germinated Chenopodium (*Chenopodium album*) flour. *Swiss Society of Food Science and Technology*, *1*, 281–287.

Jarup, L. (2003). Hazards of heavy metal contamination. *British Medical Bulletin*, *68*, 167-182.

Jennifer, A. and Kanjana, K. (2018). Effect of Seaweed Based Biscuit Supplementation on Anthropometric Profile of Malnourished Children Residing at Tuticorin. *A Journal of Science and Technology*, *4*(2), 2349–5456.

Jensen, A. (1969). Tocopherol content of seaweed and seaweed meal. II. Individual, diurnal and seasonal variations in some Fucaceae. *Journal of the Science of Food and Agriculture*, *20*, 454 – 458.

Juneng, L. and Tangang, F. T. (2008). Level and source of predictability of seasonal rainfall anomalies in Malaysia using canonical correlation analysis. *International Journal of Climatology*, *28*, 1255–1267.

Kaladharan, P. (2006). Animal feeds from Seaweeds. *National Training Workshop on Seaweed Farming and Processing for Food*, 83-90.

Kaliaperumal, N. (2003). *Products from seaweeds*. *National Seminar on Marine Biodiversity as a Source of Food Medicine*, *3*, 33–42.

Kalimuthu, S., Kaliaperumal, N. and Ramaligam, J. R. (1991) Standing crop, algin and mannitol of some alginophytes of Mandapam coast, *Journal of the Marine Biological Association of India*, *33*(1and2), 170-174.

Kaaria, P., Wakibia, J., Matiru, V. Ndung'u, M. and Bii, C. (2015). Antimicrobial Screening of Marine Endophytes and Epiphytes Isolated from Marine Algae of Kenyan Indian Ocean, *Journal of Applied and Environmental Microbiology*, *3*(3), 70-74.

KDHS (2008). *Kenya Demographic and Health Survey*. National Council for Population and Development, Central Bureau of Statistics and Ministry of National Planning and Development, Nairobi, Kenya.

Khairy, H. M. and El-Shafay, S. M. (2013). Seasonal variations in the biochemical composition of some common seaweed species from the coast of Abu Qir Bay, Alexandria, Egypt. *Oceanologia*, *55*(2), 435–452.

Khan, S. I. and Satam, S. B. (2003). Seaweed mariculture: scope and potential in India. *Aquaculture Asia*, *4*(4), 26-28.

Khan S. K., Hoq, E., Haque, A., Islam, M. and Hoque, M. (2016). Nutritional evaluation of some seaweeds from the Bay of Bengal in contrast to inland fishes of Bangladesh *Journal of Environmental Science, Toxicology and Food Technology*, *10*(11), 59-65.

Kilinç, B., Cirik, S., Turan, G., Tekogul, H. and Koru, E. (2013). *Seaweeds for Food and Industrial Applications*, pp 735-748. https://dx.doi.org/10.5772/53172.

Kilinç, B., Turan, G. and Tekogul, H. (2011). Marinate production and sensory evaluation in different formulations with cultured seaweed (*Ulva rigida*). *16th National Fisheries Symposium* (25th-27th October, 2011), 272-273 (in Turkish).

Kim, J. K., Kraemer, G. P. and Yarish, C. (2014). Field scale evaluation of seaweed aquaculture as a nutrient bioextraction strategy in Long Island Sound and the Bronx River Estuary. *Aquaculture*, *433*, 148-156.

Kimiywe, E. and Simiyu, N. W. (2009). The extent of dietary supplements use by male rugby players in Kenya, *Journal of Applied Biosciences*, *22*, 1306-1311.

Krishnaiah, D., Sarbatly, R., Prasad, D. M. R. and Bono, A. (2008). Mineral content of some seaweeds from Sabah's South China Sea. *Asian Journal of Scientific Research, 1*(2), 166-170.

Kubanek, J., Jensen, P. R., Keifer, P. A., Sullards, C., Collins, D. O. and Fenical, W. (2003). Seaweed Resistance to Microbial Attack: A targeted chemical defence against marine fungi. *Proceedings of the National Academy of Sciences of the United States of America*, *100*(12), 6916-6921.

Kumar, A., Krishnamoorthy, E., Devi, H. M., Uchoi, D., Tejpal, C. S. and Zynudheen, A. A. (2018). Influence of sea gapes (*Caulerpa racemosa*) supplementation on physical, functional and anti-oxidant properties of semi-sweet biscuits. *Journal of Applied Phycology*, *30*(2), 1393-1403.

Lahaye, M. and Thibault, J. F. (1990). *Chemical and physio-chemical properties of fibers from algal extraction by-products*. Cambridge: Royal Society of Chemistry, 68-72.

Lahaye, M. (1991). Marine algae as sources of fibres: Determination of soluble and insoluble dietary fiber contents in some sea vegetables. *Journal of the Science of Food and Agriculture*, *54*, 87-594.

Lahaye, M. (2001). Chemistry and physico-chemistry of phycocolloids, *Cahiers de Biologie Marine*, *42*, 137-157.

Lapointe, B. E. and Duke, S. C. (1984). Biochemical Strategies for Growth of *Gracilaria tikvahiae* (Rhodophyta) in Relation to Light Intensity and Nitrogen Availability. *Journal of Phycology*, *20*, 488-495.

Larrea-Marín, M. T., Pomares-Alfonso, M. S., Gómez-Juaristi, M., Sánchez-Muniz, F. J. and de la Rocha, S. R. (2010). Validation of an ICP-OES method for macro and trace element determination in *Laminaria* and *Porphyra* seaweeds from four different countries. *Journal of Food Composition and Analysis*, *23*, 814–820.

Leenstra, T., Kariuki, S. K., Kurtis, J. D. and Oloo, A. J. (2004). Prevalence and severity of anemia and iron deficiency: cross-sectional studies in adolescent schoolgirls in western Kenya. *European Journal of Clinical Nutrition*, *58*, 681-691.

Le Tutour, B. (1990). Antioxidative activities of algal extracts, synergistic effect with vitamin E. *Phytochemistry*, *29*(12), 3759-3765.

Levine, I. (2016). Algae: a way of life and health. In Seaweed in Health and Disease Prevention (Fleurence, J. & Levine, I. A., editors), London: Elsevier Academic Press, pp 1–5.

Lobban, C. S. and Harrison, P. H. (1994). *Seaweed ecology and physiology*. New York, NY: Cambridge University Press.

Lourenço, S. O., Barbarino, E., Lavín, P. L., Marquez, U. M. L. and Aidar, E. (2004). Distribution of intracellular nitrogen in marine microalgae: calculation of new nitrogen-to-protein conversion factors. *European Journal Phycology, 39,* 17-32.

Mabeau S. and Fleurence J. (1993). Seaweed in food products: bio-chemical and nutritional aspects. *Trends in Food Science and Technology, 4,* 103-107.

MacArtain, P., Gill, C. I., Brooks, M., Campbell, R. and Rowland, I. R. (2007). Nutritional value of edible seaweeds. *Nutrition Reviews, 65*(12), 535-43.

Madlener, J. C. (1977). *Sea vegetable Book*, New York NY: Carlkson N. Potter Inc.

Malaysia, (2009). Climate change scenarios for Malaysia 2001–2099. *Malaysia Meteorological Department: Petaling Jaya*, Malaysia.

Manley, D. (2011). *Manley's technology of biscuits, crackers and cookies*. London: Elsevier Ltd.

Mannar M. G. and Sankar, R. (2004). Micronutrient fortification of foods-rationale, application and impact. *Indian Journal of Pediatrics*, *71*, 997-1002.

Manivannan, K., Thirumaran, G., Karthikai Devi, G., Anantharaman, P. and Balasubramanian, T. (2009). Proximate composition of a different group of seaweeds from Vedalai Coastal waters (Gulf of Mannar): Southeast Coast of India. *Middle-East Journal of Scientific Research*, *4*(2), 72-77.

Mariamenatu, A. H. and Abdu, E. N. (2021), Overconsumption of Omega-6 Polyunsaturated Fatty Acids (PUFAs) versus Deficiency of Omega-3 PUFAs in Modern-Day Diets: The Disturbing Factor for Their “Balanced Antagonistic Metabolic Functions” in the Human Body. *Journal of Lipids, Agriculture and Healthcare*, *7*(12), pp 37-45.

Marinho-Soriano, E., Fonseca, P. C., Carneiro, M. A. A. and Moreira, W. S. C. (2006). Seasonal variation in the chemical composition of two tropical seaweeds. *Bioresource Technology, 97*(18), 2402-2406.

Mason, J. B., Lotfi, M., Dalmiya, N., Sethuraman, K., Deitchler, M., Geibel, S., Gillenwater, K., Gilman, A., Mason, K. and Mock, N. (2001). The micronutrient report: Current progress in the control of vitamin A, iodine, and iron deficiencies. Ottawa: International Development Research Centre.

Matanjun, P., Mohamed, S., Mustapha, N. M., Muhammad, K. and Ming, C. H. (2008). Antioxidant activities and phenolics content of eight species of seaweeds from north Borneo. *Journal of Applied Phycology, 20*, 367–373.

Matanjun, P., Mohamed, S., Mustapha, N. M. and Muhammad, K. (2009). Nutrient content of tropical edible seaweeds, *Eucheuma cottonii*, *Caulerpa lentillifera* and *Sargassum polycystum*. *Journal of Applied Phycology, 21,* 75-80.

McDermid, K. J. and Stuercke, B. (2003). Nutritional composition of edible Hawaiian seaweeds. *Journal of Applied Phycology*, *15*, 513–524.

McHugh, D. J. (2003). *A Guide to the Seaweed Industry.* FAO Fisheries Technical Paper 441, Rome, 105 pp

Meenakshi, J. V., Johnson, N. L., Manyong, V. M., Degroote, H. and Javelosa, J. (2010). How Cost-Effective is Biofortification in Combating

Micronutrient Malnutrition? An Ex Ante Assessment. *World Development*, *38*, 64-75.

Mendis, E. and Kim, S. K. (2011). Present and future prospects of seaweeds in developing functional foods. In advances in food and nutrition research, Volume 64: Marine Medicinal Foods; Implications and Applications, Macro and Microalgae. *Elsevier Inc.*, 1-13.

Mondal, A. and Datta, A. K. (2008). Bread baking - A review. *Journal of Food Engineering*, *86*(4), 465-474.

Moorjani, S. A. (1977). The Ecology of Marine Algae of the Kenya coast. PhD thesis, Kenya: University of Nairobi.

Mourandi-Givernaud, A., Givernaud, T., Morvan, H. and Cosson, J. (1993). Annual variations of the biochemical composition of *Gelidium latifolium* (greville) Thuret et Bornet; *Hydrobiologia*. *260/261*, 607–612.

Msuya, F. E. (2006). Seaweed farming as a potential cluster. In Mwamila BLM, Temu AK (eds.), Proceedings of the innovation systems and clusters programme in Tanzania (ISCP-Tz): cluster initiative launching workshop, Dar es Salaam, Tanzania, February 9, 2006. pp 102–113.

Msuya, F. E. (2013). Social and economic dimensions of carrageenan seaweed farming in the United Republic of Tanzania. In Valrderrama, J. C., Hishamunda, No, and Ridller, N. (eds.). Social and economic dimensions of carrageenan seaweed farming, pp. 115-146. Fisheries and Aquaculture Technical Paper No. 580. Rome, Italy: FAO. 204 pp.

Msuya, F. E, Buriyo, A., Omar, I., Pascal, B., Narrain, K., Ravina, J. J., Mrabu, E. and Wakibia, J. G. (2014). Cultivation and utilization of red seaweeds in the Western Indian Ocean (WIO) Region. *Journal of Applied Phycology*, *26*(5), 2135-2141.

Muraguri, E. N., Wakibia, J. G. and Kinyuru, J. N. (2016). Chemical Composition and Functional Properties of Selected Seaweeds from the Kenya coast. *Journal of Food Research, 5*(6), 114 -125.

Murcia, M. A., López-Ayerra, B., Martínez-Tomé, M. and García-Carmona, F. (2000). Effect of industrial processing on chlorophyll content of broccoli. *Journal of the Science of Food and Agriculture, 80*, 1447–1451.

Murugaiyan, K., Narasimman, S. and Anatharaman, P. (2012). Proximate composition of marine macroalgae from Seeniappa Dharka, Gulf of Mannar region, Tamil Nadu. *International Journal of Research in Marine Sciences*, *1*(1), 1-3.

Mutai, C. C. and Ward, M. N. (2000). East African rainfall and the tropical circulation/convection at inter-annual and intra-seasonal timescales. *Journal of Climate, 12*, 3915-3939.

Muthuraman, B. and Ranganathan, R. (2004). Biochemical studies on some green algae of Kanyakumari coast. *Seaweed Research and Utilisation*, *26*(1-2), 69-71.

Mwalugha, H. M., Wakibia, J. G., Kenji, G. M. and Mwasaru, M. A. (2015). Chemical composition of common seaweeds from the Kenya coast. *Journal of Food Research*, *4*(6), 28-38.

Nagi, H. P. S., Kaur, J., Dar, B. N. and Sharma, S. (2012). Effect of storage period and packaging on the shelf life of cereal bran incorporated biscuits. *American Journal of Food Technology*, *7*(5), 301–310.

National Research Council. (1989). Nutrient requirements (pp 655 – 676) Washington, DC: National Academy Press.

Neori, A. (2016). Can sustainable mariculture match agriculture's output? *The Global Aquaculture advocate*. pp. 1-6.

Neto, R., Marçal, C., Queirós, A., Abreu, H., Silva, A. and Cardoso, S. (2018). Screening of *Ulva rigida*, *Gracilaria* sp., *Fucus vesiculosus* and *Saccharina latissima* as functional ingredients. *International Journal of Molecular Sciences*, *19*, 2987.

Nisizawa, K., Noda, H., Kikuchi, R. and Watanabe, T. (1987). The main seaweeds in Japan. *Hydrobiologia*, *151/152*, 5–29.

Noguchi, T., Matsu, T., Miyazawa, K., Asakawa, M., Iijima, N., Shida, Y., Fuse, M., Hosaka, Y., Kirigaya, C., Watabe, K., Usui, S. and Fukagawa, A., (1994). Poisoning by the red alga "Ogonori" (*Gracilaria verrucusa*) on the Nojima Coast, Yokohama, Kanagawa Prefecture, Japan. *Toxicon*, *32*, 1533–1538.

Norziah, M. H. and Ching, C. Y. (2000). Nutritional composition of edible seaweed *Gracilaria changgi*, *Food Chemistry, 68,* 69-76.

Novaczek, I. (2001). *A guide to the Common and Edible and Medicinal Sea Plants of the Pacific Island.* Suva, Fiji: University of the South Pacific.

Ohno, M. (1993). Cultivation of the green alga, *Monostroma* and *Entromorpha* "aonori" (pp. 7-15). In Ohno, M. and Critchley, A. (eds.). (1993). *Seaweed Cultivation and Marine Ranching*. Yokosuka, Japan: Japan Internation Cooperation Agency.

Ohno, M. and Critchley, A. (1993). *Seaweed Cultivation and Marine Ranching*. Yokosuka, Japan: Japan Internation Cooperation Agency.

Ometto, F., Steinhovden, K. B., Kuci, H., Lunnbäck, J., Berg, A., Karlsson, A., Handå, A., Wollan, H. and Ejlertsson, J. (2018). Seasonal variation of elements composition and biomethane in brown macroalgae. *Biomas Bioenergy*, *109*, 31–38.

Orduna-Rojas, J., Robledo, D. and Dawes, C. J. (2002). Studies on the Tropical Agarophyte *Gracilaria cornea* J. Agardh (Rhodophyta, Gracilariales) from Yucatan, Mexico. I. Seasonal Physiological and Biochemical Responses; *Botanica Marina*, *45*, 453–458.

Ortiz, J., Romero, N., Robert, P., Araya, J., Lopez-Hernández, J., Bozzo, C. E., Navarrete, C. E., Osorio, A. and Rios, A. (2006). Dietary fiber, amino acid, fatty acid and tocopherol contents of the edible seaweeds *Ulva lactuca* and *Durvillaea antarctica*. *Food Chemistry, 99*, 98–104.

Ottaway, P. B. (2008). *Food fortification and supplementation: technological, safety and regulatory aspects*. Cambridge: Woodhead Publishing Ltd.

Ovando-Martinez, M., Sáyago-Ayerdi, S., Agama-Acevedo, E. E., Goñi, I. A. and Bello-Pérez, L. A. (2009). Unripe banana flour as an ingredient to increase the undigestible carbohydrates of pasta. *Food Chemistry*, *113*, 121–126.

Oyieke, H., (1996). Marine Biodiversity in Eastern Africa: Research and Sustainable Utilization. Nairobi: Centre for biodiversity, National Museums of Kenya. pp 378-385

Padula, M. and Boiteux, S. (1999). Photodynamic DNA damage induces by phycocyanin and its repair in *Saccharomyces cerevisiae*. *Brazilian Journal of Medical and Biological Research*, *32*, 1063-1071.

Paiva, L., Lima, E., Neto, A. I., Marcone, M. and Baptista, J. (2016). Health-promoting ingredients from four selected Azorean macroalgae. *Food Research International*, *89*, 432–438.

Patel, M. M. and Rao, G. V. (1996). Effect of untreated, roasted, and sprouted black gram (*Phaseolus mungo*) flours on the Physico-chemical and biscuit (cookie) making characteristics of soft wheat flour. *Journal of Cereal Science, 22*, 285–291.

Paull, R. E. and Chen, N. J. (2008). Postharvest handling and storage of the edible red seaweed *Gracilaria. Postharvest Biology and Technology*, *48*, 302-308.

Pavia, H. and Brock. E. (2000). Extrinsic factors influencing phlorothannin production in the brown alga *Ascophyllum nodosum*. *Marine Ecology Progress Series*, *193*, 285-294.

Pavia, H., CelVin, G., Lindgren, A. and Aberg, P. (1997). Effects of UV-radiation and simulated herbivory on phlorothannins in the brown alga *Ascophylum nodosum*. *Marine Ecology Progress Series*, *157*, 139-146.

Payri, C., N'Yeurt, A. D. R. and Orempulier, J. (2000). Algae of French Polynesia. *Au Vent des Illes*, pp 320 (in French).

Pellizzari, F. and Reis, R. P. (2011). Seaweed cultivation on the southern and southeastern Brazilian coast. *Revista Brasileira de Farmacognosia*, *21*, 305–312.

Pereira, R. and Yarish, C. (2008). Mass production of marine macroalgae. In Ecological Engineering, Volume. 3 of Encyclopedia of Ecology (Jørgensen, S.E. & Fath, B.D., editors), (pp. 2236–2247). Oxford: Elsevier.

Pereira, R. and Yarish, C. (2010). The role of Porphyra in sustainable culture systems: Physiology and Applications. In: Israel, A., Einav, R., (eds.), *Role of Seaweeds in a Globally Changing Environment* (pp. 339-354). New York NY: Springer Publishers.

Pérez, R., Kaas, R., Campello, F., Arbault, S. and Barbaroux, O. (1992). *The cultivation of seaweeds around the world*. Plouzane, France: Service de la Documentation et des Publications (in French).

Pettersson-Löfquist, P. (1995). The development of open-water algae farming in Zanzibar: Reflections on the socioeconomic impact. *Ambio*, *24*(7-8), 487-91

Pfeiffer, C. C. and Braverman, E. R. (1997) Zinc, the brain, and behaviour. *Biological Psychology*, *17,* 513–30.

Phang, S. M., Yeong, H. Y. and Lim, P. E. (2019). The seaweed resources of Malaysia, *Botanica Marina*, *62,* 265 pp.

Piovetto, L. and Peiffer, P. (1991). Determination of sterols and diterpenoids from brown algae (Cystoseiraceae). *Journal of Chromatography*, *588,* 99-105.

Polat, S. and Ozogul, Y. (2009). Fatty acid, mineral and chemical composition of some seaweeds from the North-eastern Mediterranean Coast. *Italian Journal of Food Science, 21*(3), 317-324.

Portugal, T. R., Ladines, E. O., Ardena, S. S., Resurreccion, L., Medina, C. R. and Matibag, P. M. (1983). Nutritive value of some Philippine seaweeds, part 2: proximate amino acid and vitamin composition. *Philippian Journal of Nutrition*, *36*(4), 166–172.

Prasad, A. S. (1991). Discovery of human zinc deficiency and studies in an experimental model. *American Journal of Clinical Nutrition*. *53*, 403 pp

Prasad, K. N. (2011). *Micronutrients in Health and Disease*. Boca Raton: CRC Press.

Price, A. R. G., Vincent, L. P. A., Venkatachalam, A. J., Bolton, J. J. and Basson, P. W. (2006). Concordance between different measures of biodiversity in Indian Ocean macroalgae. *Journal of Experimental Marine Biology and Ecology, 319,* 85–91.

Pyler, E. J. (1982). *Baking science and technology* (pp. 367–395). Chicago, IL: Siebel.

Ramakrishnan, U. (2002). Prevalence of micronutrient malnutrition worldwide. *Nutrition Reviews, 60*, 546-552.

Ranjana, S., Gurmukh, S., Chaauhan, G. S., Singh, R. and Singh, G. (1998). Effect of incorporation of defatted soya flour on the quality of biscuits. *Indian Journal of Food Science and Technology*, *35*(4), 355-357.

Rao, B. D. and Bhargavi, G. (2017). Technology Involved in Quality of Biscuits: Influence of Factors and Impact on Processing – A Critical Review. *International Journal of Pure and Applied Biosciences*, *5*(4), 532-542

Rao, P. V. S., Mantri, V. A. and Ganesan, K. (2007). Mineral composition of edible seaweed *Porphyra vietnamensis*. *Food Chemistry, 102*(1), 215–218.

Ratana-arporn, P. and Chirapart, A. (2006). Nutritional evaluation of tropical green seaweeds *Caulerpa lentillifera* and *Ulva reticulatum*. *Kasetsart Journal (Natural Science)*, *40*(Suppl.), 75–83.

Remirez, D., Gonzalez, A., Merino, N., Gonzalez, R., Ancheta, O., Romay, C. and Rodriguez, S. (1999). Effect of phycocyanin in Zymosan-induced arthritis in mice-phycocyanin as an antiarthritic compound. *Drug Development Research, 48*, 70-75.

Renaud, S. M. and Luong-Van, T. T. (2006). Seasonal variation in the chemical composition of tropical Australian marine macroalgae. *Journal of Applied Phycology*, *18*, 381-387.

Rohani-Ghadikolaei, K., Abdulalian, E., Ng, W. K. (2012). Evaluation of the proximate, fatty acid and mineral composition of representative green, brown and red seaweeds from the Persian Gulf of Iran as potential food and feed resources. *Journal of Food Science and Technology*, *49*(6), pp. 774-780.

Rolfes, S. R., Pinna, K. and Whitney, E. (2008). Understanding Normal and Clinical Nutrition (8th Ed.) (pp. 442-450). Belmont: Wadsworth.

Rosemberg, G. and Ramus, J. (1982) Ecological growth strategies in the seaweeds *Gracilaria follifera* (Rhodophyceae) and *Ulva* sp. (Chlorophyceae): soluble nitrogen and reserve carbohydrates. *Marine Biology*, *66*, 251–259.

Rotem, A., Roth-Bejeranu, N. and Arad, S. M. (1986). Effect of controlled environmental conditions on starch and agar contents of *Gracilaria* sp. (Rhodophyceae). *Journal of Phycology*, *22*, 117–121.

Ruperez, P. (2002). Mineral content of edible marine seaweeds. *Food Chemistry*, *79*, 23–36.

Rushdi, M. I., Abdel-Rahman, I. A. M., Attia, E. Z., Abdelraheem, W. M., Saber, H., Madkour, H. A., Amin, E., Hassan, H. M. and Abdelmohsen, U. R. (2020). A review on the diversity, chemical and pharmacological potential of the green algae genus *Caulerpa*. *South African Journal of Botany*, *132*, pp. 226-241.

Sanchez-Machado, D. I., Lopez-Cervantes, J., Lopez-Hernandez, J. and Paseiro-Losada, P., (2004). Fatty acids, total lipid, protein and ash contents of processed edible seaweeds. *Food Chemistry*, *85*(3), 439–444.

Sandstead, H. H. (1986). Nutrition and brain function: trace elements. *Nutrition Reviews*, *44*, 37–41.

Santelices, B., Bolton, J. J. and Meneses, I. (2009). Marine Algal Communities. In: Whitman, J.D., Roy, K. (Eds.), Marine Macroecology. *Chicago University Press*. pp 153-191.

Schuhmacher, M., Domingo, J. L., Llobet, J. and Corbella, J. (1993). Dietary intake of copper, chromium and zinc in Terragona Province, Spain. *Science of the Total Environment*, *132*, 3 – 11.

Siddique, M. A. M., Aktar, M. and Mohd Khatib, M. A. (2013). Chemical composition and amino acid profile of two red seaweeds (*Hypnea pannosa* and *Hypnea musciformis*) collected from St. Martin's Island, Bangladesh. *Journal of Fisheries Sciences*, *7*(2), 178-186.

Siekmann, J. H., Allen, L. H., Bwibo, N. O., Demment, M. W., Murphy, S. P. and Neumann, C. G. (2003). Kenyan School Children have Multiple Micronutrient Deficiencies, but Increased Plasma Vitamin B-12 is the Only Detectable Micronutrient Response to Meat or Milk Supplementation. *Journal of Nutrition*, *133*(11S2), 3972S-3980S.

Shpigel, M. (2013). Mariculture mariculture systems, integrated land-based. In Sustainable Food Production (Christou, P., Savin, R., Costa-Pierce, B.A., Misztal, I. & Whitelaw, C.B.A., editors), (pp 1111–1120). NewYork, NY: Springer.

Siddhanta, A. K., Sanandiya, N. D., Chejara, D. R., Kondaveeti, S. (2015). Functional modification mediated value addition of seaweeds polysaccharides – a perspective. *Royal Society of Chemistry*, *5*, 59226-59239.

Silky, M. P. G. and Tiwari, A. (2014). Development of high protein biscuits using pigeon pea broken flour. *International Journal of Engineering and Innovative Technology*, *4*, 84–89.

Silva, P. C., Basson, P. W. and Moe, R. L., (1996). *Catalogue of the Benthic Marine Algae of the Indian Ocean*. University of California Press, Berkeley, pp. 1259.

Southgate, D. A. T. (1990). Dietary fibre and health (pp. 282-284). In Dietary fibre: Chemical and Biological Apects. Cambridge, UK: The Royal Society of Chemistry.

Stein, J. R. and Borden, C. A. (1984). Causative and beneficial algae in human disease conditions: a review. *Phycologia*, *23*, 485-501.

Stephenson, T. A. and Stephenson, A. (1949). The universal features of zonations between tide marks on rocky coasts. *Journal of Ecology*, *37*, 289–305.

Suo, R. & Wang, Q. (1992). Laminaria culture in China. *Infofish International*, *1*, pp. 40–42.

Tabarsa, M., Rezaei, M., Ramezanpour, Z. and Waaland, J. R. (2012). Chemical compositions of the marine algae *Gracilaria salicornia* (Rhodophyta) and *Ulva lactuca* (Chlorophyta) as a potential food source. *Journal of Science, Food and Agriculture*, *92*, 2500–2506.

Tanaka, J. and Nakamura, T. (2004). *Japanese Seaweeds.* Tokyo, Japan: Technical Reports (in Japanese).

Teas, J., Pino, S., Critchley, A. T. and Braverman, L. E. (2004). Variability of iodine content in common commercially available edible seaweeds. *Thyroid*, *14*(10), 836–841.

Thoma, T. (1997). *Cultivation of the brown alga, Cladosiphon okamuranus 'okinawa-Mozuku' in Seaweeds Cultivation and Marine Ranching* (pp. 51-56). In Ohno, M. and Cricthely, A. (Eds). Yokosuka, Japan: Japan Internation Cooperation Agency.

Tokida, J. (1954). The Marine Algae of Southern Sagtlalioo. *Reprinted from the Memoirs of the Faculty of Fisheries Hokkaido University*. *2*(1), pp 264.

Trono, G. C. (1999). Diversity of seaweed flora of the Philippines and its utilization. *Hydrobiologia, 389/399*, 1-6.

Trono, G. C. and Toma, T. (1993). Cultivation of the green alga, *Caulerpa lentillifera. In Ohno and Critchley*. pp 17-23.

Tsutsui, I., Huybh, Q. N., Nguyen, H. D., Arai, S. and Yoshida, T. (2005). *The Common Marine Plants of Sothern Vietnam* (pp. 250). Kochi: Japan Seaweed Association.

Tulchinsky, T. H. (2010). Micronutrient Deficiency Conditions: Global Health Issues. *Public Health Reviews, 32*, 243-255.

Turan, G., Tekogul, H., Kilinç, B. and Cirik, Ş. (2011). Sensory evaluation of seaweed breads prepared with in-house cultured sea lettuce (*Ulva rigida*) and determination of shelflife (pp. 275). *16th National Fisheries Symposium*, Antalya, Turkey. (in Turkish).

Uchendu, F. N. (2011). Micronutrient Malnutrition, A Tragedy to Childhood Growth and Education. *Global Journal of Medical Research, 11*, 26-34.

UNICEF (2009). *Tracking progress in child and maternal nutrition. A survival and development priority*. New York, NY: United Nations Children's Fund. pp 1-99.

United States Department of Agriculture (USDA) (2016). National Nutrient Database for Standard Reference Release 28. Retrieved from https://ndb.nal.usda.gov/ndb/search/list

Unnevehr, L., Pray, C. and Paarlberg, R. (2007). Addressing Micronutrient Deficiencies: Alternative Interventions and Technologies. *AgBioForum, 10*, 124-134.

Untoro, J., Muslimatun, S. and Karyadi, E. (2002). Micronutrient supplementation and its relationship to nutritional status, health and diseases. *Asia Pacific Journal of Clinical Nutrition*, *11*, 355-359.

Vallejo, F., Tomás-Barberán, F. A. and Garcia-Viguera, C. (2002). Glucosinolates and vitamin C content in edible parts of broccoli florets after domestic cooking. *European Food Research and Technology*, *215,* 310–316.

van Hoof, L. A. and Steins, N. (2017). Mission Report Kenya – Scoping Mission Marine Fisheries Kenya (pp 1-137). Ijmuiden: Wageningen University & Research.

Varea, A., Malpeli, A., Etchegoyen, G., Vojkovic, M. and Disalvo, L. (2011). Short-term evaluation of the impact of a food program on the micronutrient nutritional status of Argentinean children under the age of six. *Biological Trace Element Research*, *143*, 1337-1348.

Veenemans, J. (2011). Effect of preventive supplementation with zinc and other micronutrients on malaria and diarrhoeal morbidity in African children. PhD thesis, Netherlands: Wageningen University.

Vizetto-Duarte, C., Custódio, L., Barreira, L., Moreira da Silva, M., Rauter, A. P., Albericio, F. and Varela, J. (2016). Proximate biochemical composition and mineral content of edible species from the genus *Cystoseira* in Portugal. *Botanica Marina*, *59*(4), 251-257

Wachira, S. W. (2011). *Knowledge and Usage of dietary supplements and dietary patterns of gym users in Nairobi*. MSc thesis, Kenya: University of Nairobi.

Wada, K., Nakamura, K. and Tamai, Y. (2011). Seaweed intake and blood pressure levels in healthy pre-school Japanese children. *Nutrition Journal*, *10*(83), 1-7.

Wakibia, J. G. (2005). *Linking biology and sustainable livelihoods to the proposed establishment of community based euchemoid farming in Southern Kenya*. PhD thesis, South Africa: University of the Western Cape.

Wakibia, J. G., Bolton, J. J., Keats, D. W. and Raitt, L. M. (2006). Factors influencing growth rates of three commercial eucheumoids at coastal sites in southern Kenya. *Journal of Applied Phycology*, *49*, 208–215.

Wakibia, J. G., Ochiewo, J. and Bolton, J. J. (2011). Economic Analysis of Eucheumoid Algae Farming in Kenya. *Western Indian Ocean Journal of Marine Science, 10* (1), 13-24.

Walker, J. M. (1988). Regulation by other countries in foods and the human environment, Proceeding, No. 2, Cadmium Accumulation in Australian Agriculture (pp. 176-185). National Symposium, Canberra, Australia, 1st – 2nd March 1988. Canberra: Australian Government Publishing Service.

WHO (1995). *Environmental Health Criteria* (Vol. 165). Geneva, Switzerland: World Health Organization.

WHO (2001). *Iron deficiency anaemia: assessment, prevention and control. A guide for programme managers*. Geneva, Switzerland: World Health Organization.

WHO (2016). *WHO Guideline: fortification of maize flour and corn meal with vitamins and minerals*. Geneva, Switzerland: World Health Organization.

Wijesinghe, W. A. J. P. and Jeon, Y-J. (2012). Enzyme-assistant extraction (EAE) of bioactive components: a useful approach for recovery of industrially important metabolites from seaweeds: a review. *Fitoterapia, 83*, 6–12.

Winberg, P. C. (2008). Using Algae in the 21st Century - Novel Opportunities in a Changing World. Report from the 11th International Conference on Applied Phycology, Galway, Ireland, 2008. (Rural Industries Research and Development Corporation, Publication no. 08/157).

Wong, C. L., Liew, J., Yusop, Z., Ismail, T., Venneker, R. and Uhlenbrook, S. (2016). Rainfall Characteristics and Regionalization in Peninsular Malaysia Based on a High Resolution Gridded Data Set. *Water, 8*, 500-524.

Wong, K. H. and Cheung, P. C. K. (2000). Nutritional evaluation of some subtropical red and green seaweeds - Part I – Chemical composition, amino acid profiles and some physico-chemical properties. *Food Chemistry, 71*(4), 475-482.

Xia, L., Huang, H., Fan, Z., Hu, D., Zhang, D., Khan, A. S., Usman, M. and Pan, L. (2019). Hierarchical macro-/meso-/microporous oxygen-doped carbon derived from sodium alginate: A cost-effective biomass material for binder-free supercapacitors. *Material and Design*, *182*, pp 1-9.

Yaich, H., Garna, H., Bchir, B., Besbes, S., Paquot, M., Richel, A., Blecker, C. and Attia, H. (2015). Chemical composition and functional properties of dietary fibre extracted by Englyst and Prosky methods from the alga *Ulva lactuca* collected in Tunisia. *Algal Research*, *9*, 65-73.

Yang, L. E., Lu, Q. Q. and Brodie, J. (2017). A review of the bladed *Bangiales* (Rhodophyta) in China: history, culture and taxonomy. *European Journal of Phycology*, *52*, 1–13.

Yarish, C. and Wamukoya, G. (1990). Seaweeds of economic importance: field survey and future prospects. *Hydrobiologia*, *204/205*, 339–346.

Zhang, D. and Hamauzu, Y. (2004). Phenolics, ascorbic acid, carotenoids and antioxidant activity of broccoli and their changes during conventional and microwave cooking. *Food Chemistry*, *88*, 503–509.

Zhang, X. C., Zheng, L. H., Liu, S. W., Tang, X. R. and Wang, J. F. (2004). Introduction to new economical seaweed, *Cladosiphon okamuranus*. *Periodical of Ocean University of China*, *34*, 807-810.

Zhu, Q., Zhang, X. and Arunakumara, K. (2009). Ultrastructure of sporophyte of *Cladosıphon okamuranus* Tokida (Ectocarpales, Phaeophyceae). *Bangladesh Journal of Botany*, *38*(2), 177-180.

Zia, Z. (1990). *Utilization of Seaweed as manure*. M.Phil thesis, Pakistan: University of Karachi.

APPENDICES

Appendix I: Sensory evaluation questionnaire for wheat-seaweed biscuits

Name: ……………………………………….....   Date: ………………………

You are provided with six (6) coded samples of biscuits. Please rate the samples (1-9) according to the scale provided below by filling in the table against each sample and attribute with 1, for Dislike extremely and 9, for Like extremely.

Kindly rinse your mouth for every sample evaluated.

Description	**Score**
Dislike extremely	1
Dislike very much	2
Dislike moderately	3
Dislike slightly	4
Neither like nor dislike	5
Like slightly	6
Like moderately	7
Like very much	8
Like extremely	9

Sample code	Colour	Hardness	Aroma	Taste	Overall consumer preference
GR51					
AX36					
PT42					
FQ08					
LZ33					
CL79					

Remarks: …………………………………………………………………………...
………………………………………………………………………………………